DOCTOR OF MEDICINE

Effects of xanthine oxidase inhibitors in pulmonary hypertension associated with chronic lung disease

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EFFECTS OF XANTHINE OXIDASE INHIBITORS IN PULMONARY HYPERTENSION ASSOCIATED WITH CHRONIC LUNG DISEASE

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List of abbreviations

6MWT	6 minute walk test
ACE	Angiotensin converting enzyme
AE	Adverse event
AHS	Allopurinol hypersensitivity syndrome
ARB	Angiotensin II receptor blocker
ATS	American Thoracic Society
BDI	Baseline dyspnoea index
BMI	Body mass index
BP	Blood pressure
CABG	Coronary artery bypass surgery
CET	Cycling endurance test
cGMP	Cyclic guanosine monophosphate
CK	Creatinine kinase
CKD	Chronic kidney disease

CMRI	Cardiac magnetic resonance imaging
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise test
CRF	Case report form
CTEPH	Chronic thromboembolic pulmonary hypertension
DLCO	Diffusion capacity for carbon monoxide
DM	Diabetes mellitus
DPLD	Diffuse parenchymal lung disease
DRESS	Drug reaction with eosinophilia and systemic symptoms
ECG	Electrocardiogram
Echo	Echocardiography
EDTA	Ethylenediaminetetraacetic acid
ERS	European Respiratory Society
ESC	European Society of Cardiologist

ESWT	Endurance shuttle walk test
ET	Endothelin-1
FBC	Full blood count
FEF	Forced expiratory flow
FEV_1	Forced expiratory volume in 1 second
FMD	Flow-mediated dilation
FVC	Forced volume capacity
FVOP	Forearm venous-occlusion plethysmography
GCP	Good Clinical Practice
GOLD	Global Initiative for chronic obstructive lung disease
GP	General Practice
GPX	Glutathione peroxidase
H_2O_2	Hydrogen peroxide
HDL-C	High-density lipoprotein cholesterol
HOCl	Hypochlorous acid

HR	Heart Rate
Hg	Mercury
Hs-Trop I	high-sensitivity troponin I
ICS	Inhaled corticosteroid
IHD	Ischaemic heart disease
ILD	Interstitial lung disease
IMP	Investigational medicinal product
IPAH	Idiopathic pulmonary arterial hypertension
IPF	Idiopathic pulmonary fibrosis
IQR	Interquartile range
IR	Ischaemic-reperfusion
ISRCTN	International Standard Registered Clinical/social sTudy Number
ISWT	Incremental shuttle walk test
JWM	Jonathan Weir-McCall
K-BILD	King’s Brief Interstitial Lung Disease

LABA	Long-acting beta agonist
LAMA	Long-acting muscarinic antagonist
LC	Louise Cabrelli (Research nurse)
LFT	Liver function test
LTOT	Long term oxygen therapy
LV	Left ventricle
LVEDV	Left ventricular end diastolic volume
LVEDVI	Left ventricular end diastolic volume index
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end systolic volume
LVESVI	Left ventricular end systolic volume index
LVF	Left ventricular failure
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVMI	Left ventricular mass index

LVSV	Left ventricular stroke volume
LVSVI	Left ventricular stroke volume index
MHRA	Medicines and Healthcare products Regulatory Agency
MI	Myocardial infarction
MCID	Minimal clinical important difference
MID	Minimal important difference
MODS	Multiple organ dysfunction syndrome
MPAP	Mean pulmonary arterial pressure
MRI	Magnetic resonance imaging
NADPH	Nicotinamide-adenine dinucleotide
NHS	National Health Service
NO	Nitric oxide
NOS	Nitric oxide synthase
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
NYHA	New York Heart Association

O_2	Oxygen
O_2^-	Superoxide
OC	OpenClinica
$OH^{\cdot}$	Hydroxyl radicals
$OONO^{\cdot-}$	Peroxynitrite
OR	Odds ratio
OS	Oxidative stress
PA	Pulmonary artery
PAP	Pulmonary artery pressure
PAH	Pulmonary arterial hypertension
PAT	Pulmonary acceleration time
PCI	Percutaneous coronary intervention
PDE-5	Phosphodiesterase-5
PEA	Pulmonary endarterectomy
PFT	Pulmonary function test

PH	Pulmonary hypertension
PH-CLD	Pulmonary hypertension associated with chronic lung disease
PH-COPD	Pulmonary hypertension in COPD
PH-ILD	Pulmonary hypertension in ILD
PI	Principal Investigator
pKa	Acid dissociation constant
PLSC	Patrick Liu Shiu Cheong (Principal Investigator)
Pulm_PWV	Pulmonary artery pulse wave velocity
PVR	Pulmonary vascular resistance
PWA	Pulse-wave analysis
QOL	Quality of life
RA	Right atrium
RAP	Right atrial pressure
RCA	Right coronary artery
RCT	Randomised clinical trial

RHC	Right heart catheterisation
ROS	Reactive oxygen species
RR	Respiratory rate
RV	Right ventricle
RVEDV	Right ventricular end diastolic volume
RVEDVI	Right ventricular end diastolic volume index
RVEF	Right ventricular ejection fraction
RVESV	Right ventricular end systolic volume
RVESVI	Right ventricular end systolic volume index
RVF	Right ventricular failure
RVH	Right ventricular hypertrophy
RVM	Right ventricular mass
RVMI	Right ventricular mass index
RVSV	Right ventricular stroke volume
RVSVI	Right ventricular stroke volume index

SAE	Serious adverse event
SaO_2	Oxygen saturation
SD	Standard deviation
SEM	Standard error of mean
SGRQ	St George's respiratory questionnaire
SHARE	Scottish Health Research Register
SOD	Superoxide dismutases
SOP	Standard operating procedure
SPAP	Systolic pulmonary artery pressure
SPCRN	Scottish Primary Care Research Network
STEMI	ST elevation myocardial infarction
SV	Stroke volume
TASC	Tayside Medical Science Centre
TCTU	Tayside Clinical Trials Unit
TDI	Transitional dyspnoea index

TEN	Toxic epidermal necrolysis
TRV	Tricuspid regurgitation velocity
TRX	Thioredoxin
UA	Uric acid
U&E	Renal function
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase
XOR	Xanthine oxidoreductase

List of appendices

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Declaration

I hereby declare that I am the author of this thesis. All the references cited have been consulted by myself. All the data was collected and analysed by myself, including the statistics, apart from the CMRI analysis that was performed by Jonathan Weir-McCall and the blood analysis that was performed by Leslie McFarlane.

I declare that the work described in this thesis has not been previously submitted for a higher degree. The work contained in this thesis was carried out during my position as a Clinical Research Fellow in the Division of Molecular and Clinical Medicine , Ninewells Hospital & Medical School, Univeristy of Dundee between February 2015 and July 2017.

Signed

Date: 05 / 03 / 2019

Summary

Chronic lung diseases are often complicated with pulmonary hypertension (PH). This can lead to disability and poor prognosis. Oxidative stress has been implicated in the development of PH and right ventricular hypertrophy (RVH).

A possible new way to treat lung disease related pulmonary hypertension is allopurinol (a xanthine oxidase inhibitor) which decreases both uric acid and oxidative stress. We hypothesised that allopurinol could regress RVH in patients with pulmonary hypertension associated with chronic lung disease (PH-CLD).

In a double-blind, randomised controlled clinical trial, 72 patients with PH-CLD (93% diagnosed with chronic obstructive pulmonary disease and 17% with interstitial lung disease) were randomised to receive either allopurinol 300 mg twice daily or placebo for twelve months. The primary outcome was the mean change in right ventricular mass (RVM) as assessed by cardiac magnetic resonance imaging (CMRI) at twelve months. The secondary outcomes were the change in other cardiac parameters measured by CMRI, St George's Respiratory Questionnaire, Short Form 36, spirometry and six-minute walk test (6MWT).

The mean age was 71 years, the mean FEV_1 was 60% with mean resting SaO_2 of 96%. After 12 months, there was no significant change in RVM. There were also no significant changes in other cardiac parameters measured on CMRI, quality of life questionnaires, spirometry and 6MWT. Post-hoc subgroup analysis showed that allopurinol reduced RVM (allopurinol -6.16 g vs placebo

0.75 g, p = 0.02) in COPD patients with more severe airflow limitation. Patients with higher NT-proBNP (> 489 pg/ml) had a greater improvement in left ventricular ejection fraction with allopurinol 5.12 vs placebo -1.62, p = 0.02.

In summary, allopurinol had no overall impact but reduced RV mass in COPD patients with more severe airflow limitation. Further studies are warranted to assess the longer term impact of allopurinol in more severe COPD.

1. Introduction

1.1 Pulmonary hypertension

Pulmonary hypertension (PH) is defined as an increase in mean pulmonary arterial pressure (MPAP) ≥ 25 mmHg at rest as assessed by right heart catheterisation.[1] The normal MPAP at rest is 14 ± 3 mmHg, with an upper limit of normal of approximately 20 mmHg.

In the UK, it has been reported that the prevalence of pulmonary hypertension is 97 cases per million with a female-male ratio of 1.8.[1]

The diagnosis of PH requires initial high clinical suspicion based on symptoms and physical examination, and review of comprehensive set of investigations to confirm that haemodynamic criteria are met.

1.1.1 Classification of pulmonary hypertension

The causes of PH have been classified according to their clinical presentation, pathological findings, haemodynamic characteristics and treatment strategy as mentioned below[2]:

1. Pulmonary arterial hypertension (PAH)
2. Pulmonary hypertension due to left heart disease
3. Pulmonary hypertension due to lung diseases and/or hypoxia
4. Chronic thromboembolic pulmonary hypertension (CTEPH)
5. Pulmonary hypertension with unclear and/or multifactorial mechanisms

Group 1 PAH includes a variety of possible causes of PH characterised by the same pattern of vascular remodelling and are most likely to respond to PAH-specific therapy. The drugs approved to treat Group 1 PAH include phosphodiesterase-5 inhibitors, endothelin receptor antagonists, prostacyclin, calcium channel blockers and riociguat.

Group 2 consists of PH due to left heart disease (PH-LDH). PH-LDH can complicate any left heart disorder such as valvular heart diseases and congenital defects. The guidelines recommend optimisation of the treatment of the underlying condition which includes repair of valvular heart disease when indicated and aggressive therapy for heart failure with reduced systolic function.[1]

Group 3 represents PH associated with lung disease and/or hypoxia. This is discussed in more details in section 1.2.

Group 4 is a disease of obstructive pulmonary arterial remodelling as a consequence of major vessel thromboembolism. Pulmonary endarterectomy (PEA) is the treatment of choice for this group. Balloon pulmonary angioplasty is increasingly an option for patients not fit for PEA.

Group 5 includes several disorders with multiple patho-aetiologies. The mechanisms of PH are poorly understood and may include pulmonary vasoconstriction, proliferative vasculopathy, extrinsic compression, intrinsic occlusion, high-output cardiac failure, vascular obliteration and left heart failure as causes.[1]

1.1.2 Clinical presentation

The symptoms of pulmonary hypertension are non-specific and are mainly related to progressive right ventricular (RV) dysfunction. The typical symptoms are induced by exertion and include shortness of breath, fatigue, weakness, angina and syncope. Less commonly patients complain of dry cough, exercise-induced nausea and vomiting. Abdominal distension and ankle oedema develop as a consequence of progressive RV failure. Other symptoms that patients may suffer from as a result of mechanical complications of PH and of the abnormal distribution of blood flow in the pulmonary vascular bed are:

- Haemoptysis related to rupture of hypertrophied bronchial arteries.
- Hoarseness of voice caused by compression of left recurrent laryngeal nerve secondary to pulmonary arterial dilatation.
- Wheeze caused by large airway compression.
- Angina due to myocardial ischaemia caused by compression of left main coronary artery.

The physical signs that may be present in PH include left parasternal heave, an accentuated pulmonary component of the second heart sound, a pansystolic murmur of tricuspid regurgitation and a diastolic murmur of pulmonary regurgitation. In advanced disease, there are signs of right sided heart failure such as elevated jugular venous pressure, hepatomegaly, ascites, peripheral oedema and cool extremities.

1.1.3 Investigations

Electrocardiogram

The abnormalities seen on electrocardiogram (ECG) include P pulmonale, right axis deviation, RV hypertrophy, RV strain, right bundle branch block and QTc prolongation. Supraventricular arrhythmias (such as atrial flutter and atrial fibrillation) may occur in advanced disease.[3]

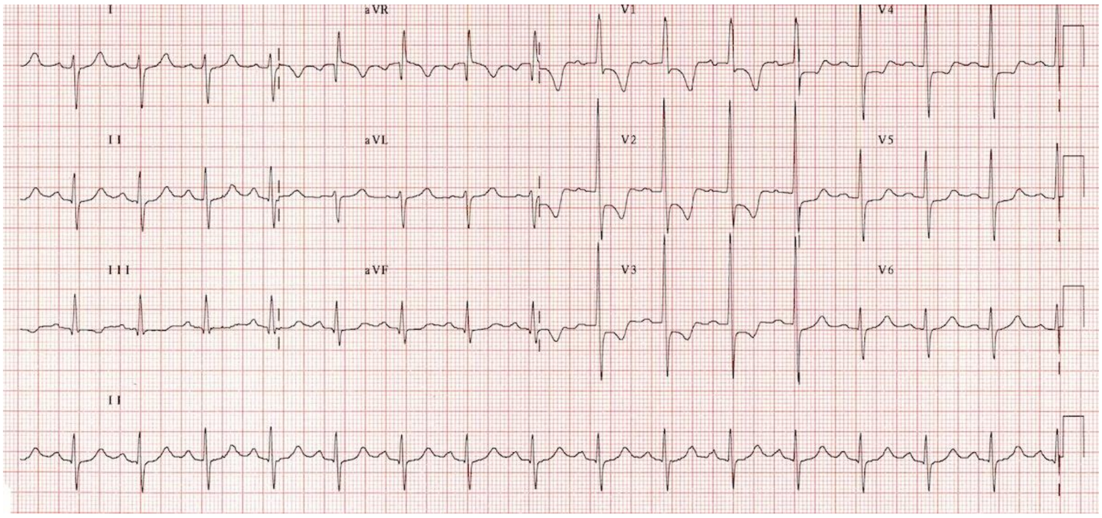

Figure 1 - Right ventricular hypertrophy: right axis deviation, right ventricular strain pattern with ST depression and T-wave inversion in V1-4

Chest radiograph

Chest radiograph can be normal. The radiographic abnormalities that are associated with PH include central pulmonary arterial dilatation, loss of peripheral blood vessels, enlargement of right atrium and right ventricle.

Figure 2 - Chest x-ray showing central pulmonary arterial dilatation

Pulmonary function tests

Patients with pulmonary hypertension have a decreased lung diffusion capacity for carbon monoxide (DLCO). Studies have show that very low DLCO (< 45% predicted) is associated with poor outcome.[4] Pulmonary function tests also can help to identify the contribution of underlying airway or parenchymal lung disease.

Echocardiogram

Transthoracic echocardiography should always be performed when PH is suspected. The echocardiographics signs suggesting pulmonary hypertension are in table 1 below.

Ventricles	Pulmonary artery	Inferior vena cava & Right atrium
Right ventricle / left ventricle basal diameter ratio > 1.0	Right ventricular outflow Dopper acceleration time < 105 ms and/or midsystolic notching	Inferior cava diameter > 21 mm with decreased inspiratory collapse (< 50% with a sniff or < 20% with quiet inspiration)
Flattening of interventricular septum (left ventricular eccentricity index > 1.1 in systole and/or diastole)	Early diastolic pulmonary regurgitation velocity > 2.2 m/s	Right atrial area (end-systole) > 18 cm^2
	PA diameter > 25 mm	

Table 1 - Echocardiographic signs of pulmonary hypertension (adapted)[1]

The systolic pulmonary artery pressure (SPAP) can be estimated by measuring the peak tricuspid regurgitation velocity (TRV) and the right atrial pressure (RAP), using the simplified Bernoulli equation [SPAP = (4 x TRV^2) + RAP].

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (CMRI) is the technique of choice and considered to be the gold standard for accurate and reproducible assessment of the RV size, morphology and function.[5-7] It also allows the non-invasive assessment of blood flow, including stroke volume, cardiac output, pulmonary arterial distensibility and right ventricular mass (RVM).[1]

Echocardiogram	**Cardiac Magnetic Resonance Imaging**
Strengths: • Easily accessible • Quick assessment • Non-invasive	Strengths: • More reliable to measure ventricular volumes and function • Non-invasive
Weaknesses: • Images dependent on patient's body habitus • Useful for screening but cannot be used reliably for individual diagnosis of PH	Weaknesses: • Not portable • Not suitable if patient suffering from claustrophobia • Patients with pacemakers and ferromagnetic appliances cannot be studied • Expensive to purchase, maintain and operate

Table 2 - Strengths and weaknesses of echocardiogram and cardiac magnetic resonance imaging

Echocardiography is recommended to be used as a screening tool when PH is suspected. However, cardiac MRI is the gold standard for assessing cardiac volumes and function.

Right heart catheterisation

Right heart catheterisation (RHC) is an invasive investigation which is performed to confirm the diagnosis of pulmonary arterial hypertension or chronic thromboembolic pulmonary hypertension. It allows the assessment of the severity of haemodynamic impairment as well as providing measurements to support treatment decisions. Cardiac catheterisation is usually performed after the completion of other investigations so that it can answer specific questions that may arise from previous investigations and avoid an unnecessary procedure. RHC is not recommended for suspected PH in patients with lung diseases.[1]

1.2 Pulmonary Hypertension due to lung diseases and/or hypoxia

Pulmonary hypertension that develops due to lung diseases has been classified in group 3.[2] Many lung diseases can lead to pulmonary hypertension (*Figure 3*). The most common lung diseases associated with PH are chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD) and combined pulmonary fibrosis and emphysema. Mild pulmonary hypertension (MPAP 25-39 mm Hg) is common in both severe ILD and severe COPD.[8] However, severe PH (MPAP> 40 mm Hg) in lung disease is uncommon.[9] The development of PH in any lung disease is accompanied by a deterioration of exercise capacity, worsening of hypoxaemia and shorter survival.[10-12] The severity of PH is usually poorly associated with the severity of the underlying lung disease.[13,14] The most common indicator for the presence of PH in patients with underlying lung disease is a disproportionally low DLCO. [13,14]

- Chronic obstructive pulmonary disease (COPD)
- Interstitial lung disease (ILD)
- Other pulmonary diseases with mixed restrictive and obstructive pattern
- Sleep-disordered breathing
- Alveolar hypoventilation disorders
- Chronic exposure to high altitude
- Developmental lung diseases

Figure 3 - Lung diseases associated with pulmonary hypertension

1.2.1 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a common condition characterised by persistent respiratory symptoms and airflow limitation. It is predicted to become the sixth leading cause of disability and the third commonest cause of death by 2020.[15]

COPD is the collective term for chronic bronchitis (regular sputum production for three months or more in two consecutive years)[16], emphysema (destruction of the lung parenchyma) and chronic obstructive airways disease. The risk factors that are associated with COPD are cigarette smoking, tobacco, marijuana[17], indoor pollution[18] from biomass cooking and heating, occupational exposures[19] due to organic and inorganic dusts, chemical agents and fumes.

Common symptoms in patients with COPD are chronic and progressive breathlessness, chronic cough, sputum production, wheezing, chest tightness and frequent chest infections. In severe disease, patients may suffer from fatigue, weight loss[20] and anorexia. Airflow limitation on spirometry is the hallmark for the diagnosis of COPD and is defined as post-bronchodilator fixed ratio of FEV_1/FVC less than 0.70.[21] The severity of airflow limitation is classified according to GOLD stage 1 to 4 (*Table 3*).

GOLD stage	FEV_1 (% predicted)
GOLD 1	≥ 80
GOLD 2	50-79
GOLD 3	30-49
GOLD 4	< 30

Table 3 - COPD GOLD stages[21]

COPD is associated with many comorbidities, the most serious and prevalent being cardiovascular disease, lung cancer, osteoporosis, muscle weakness and cachexia.[22] A large proportion of the morbidity and mortality in COPD is associated with cardiovascular complications.[23] Mannino *et al* (2013)[9] have shown in a pooled analysis of two large epidemiological studies – the Atherosclerosis Risk in Communities study[10] and the Cardiovascular Health Study[11] – including more than 20,000 adults, that the prevalence of cardiovascular disease in patients with COPD was 20-22% compared with 9% in people without COPD. Cardiovascular disease usually encompasses ischaemic heart disease, congestive heart failure, pulmonary vascular disease, coronary artery disease, peripheral vascular disease, and stroke and transient ischaemic attack.

1.2.2 Pulmonary Hypertension in COPD

COPD may be complicated by the development of pulmonary hypertension. The severity of pulmonary hypertension in COPD (PH-COPD) patients is usually mild[14] and is closely associated with patient's age and severity of airway obstruction[24]. The pulmonary arterial pressure progresses slowly over time in patients with COPD and with mild-moderate hypoxaemia.[11] The average increase in pulmonary arterial pressure is <1 mm Hg per year.[11,25]

Prevalence

The true prevalence of PH-COPD is unknown as there are no large scale population based studies and because of selection bias in studies assessing hospitalised patients.[26] In studies[14,27] that have estimated the prevalence of PH, figures of between 30% and 90% have been obtained.

Pathophysiology

The pathogenesis of PH-COPD is poorly understood, complex and multifactorial.[24,28,29] Chronic hypoxia, inflammation, pulmonary vasculature remodelling, endothelial dysfunction, loss of blood vessels, and changes in pulmonary vascular tone have been recognised as pathogenic mechanisms. These in turn contribute to increased pulmonary vascular resistance and pulmonary hypertension. Their complex interactions are illustrated in *Figure 4*.[28]

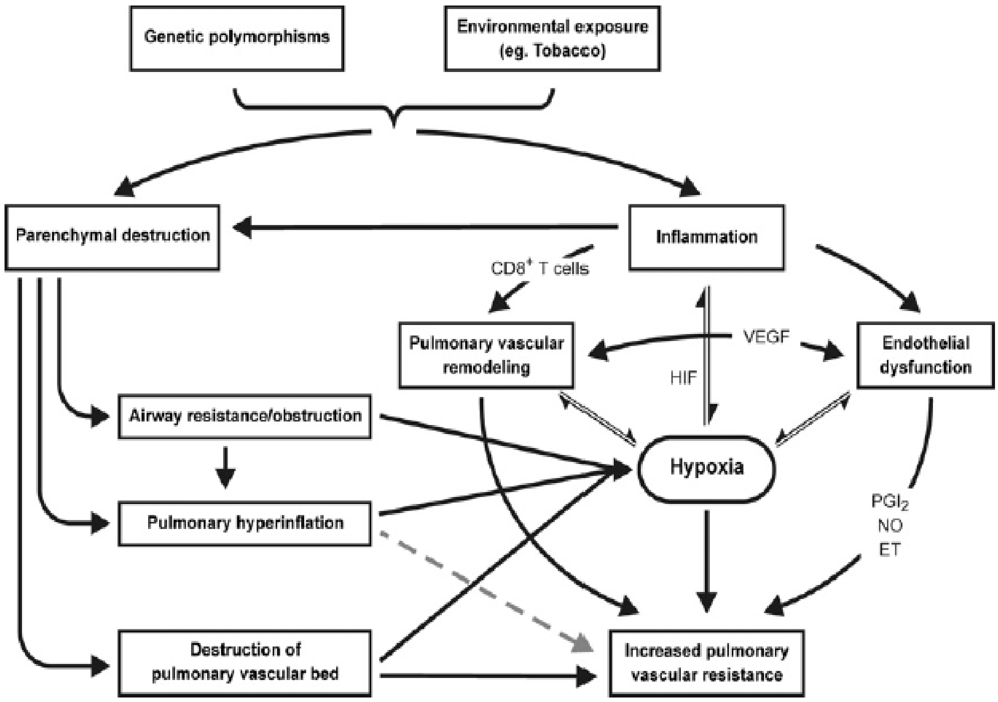

Figure 4 - Complex interactions between pathogenic mechanisms which may lead to increased pulmonary vascular resistance in patients with COPD[28]

Reprinted from *J Heart Lung Transplant*, 31(6), Wrobel JP, Thompson BR, Williams TJ. Mechanisms of pulmonary hypertension in chronic obstructive pulmonary disease: a pathophysiologic review, 557-64, Copyright (2012), with permission from Elsevier.

Alveolar hypoxia plays a key role in the development of PH-COPD. Hypoxia causes acute hypoxic pulmonary vasoconstriction of small muscular pulmonary arteries[30] and in the setting of global hypoxia this mechanism may substantially increase pulmonary vascular resistance[31] (PVR). Additionally, chronic hypoxia contributes to pulmonary vascular remodeling, resulting in intimal thickening and neo-muscularisation of small pulmonary arterioles, which also raises PVR.[32] Studies have shown that chronic hypoxia can cause endothelial dysfunction.[24]

Endothelial dysfunction plays an integral role in mediating the structural changes in the pulmonary vasculature and is caused by the altered production of various endothelial vasoactive mediators such as nitric oxide, prostacyclin, endothelin-1, serotonin and thromboxane.[33] Endothelial dysfunction has been demonstrated in COPD subjects.[34,35] It has been postulated that genetic polymorphisms, inflammation and mechanical factors are likely to play an important role in modulating the endothelial dysfunction observed in PH-COPD.[36]

Pulmonary vascular remodeling is the process of structural transformation within the pulmonary vessels. The pathologic changes identified in pulmonary vessels in patients with PH-COPD include variable medial hypertrophy, longitudinal muscle deposition, intimal hyperplasia, elastin and collagen deposition, muscularisation of pulmonary arterioles and in situ thrombosis.[28] These changes alter the pulmonary vascular responsiveness and contribute to the development of PH in COPD.

There is speculation that systemic inflammation which is a component of COPD, may promote pulmonary vascular remodeling and endothelial dysfunction in patients with COPD.[29]

Emphysematous destruction of the pulmonary vascular bed contributes to the elevation of pulmonary arterial pressure and is often listed among the pathophysiological mechanisms of PH-COPD.[13,14] The proposed mechanism is the reduction in the pulmonary capillary cross-sectional area and the reduction pulmonary blood flow.[37]

Dynamic lung hyperinflation in COPD may contribute to the development of PH through a combination of mechanisms including increased lung volume, widened intrathoracic pressure swings, cardiac effects, altered gas exchange, pulmonary vascular remodeling and endothelial dysfunction.[28]

Prognostic implications

The presence of PH in COPD is an established important prognostic factor in COPD subjects.[10,38] PH-COPD is associated with increased mortality[10], increased hospitalisations[38] and reduced exercise function[39]. Shino *et al*.[27] have demonstrated an inverse correlation between mean pulmonary artery pressure (MPAP) and/or pulmonary vascular resistance (PVR) and survival. The 5-year survival rate for COPD patients with MPAP > 25 mm Hg is only 36%.[10] PH is also a vital risk factor for hospitalisation caused by acute exacerbations of COPD.[40]

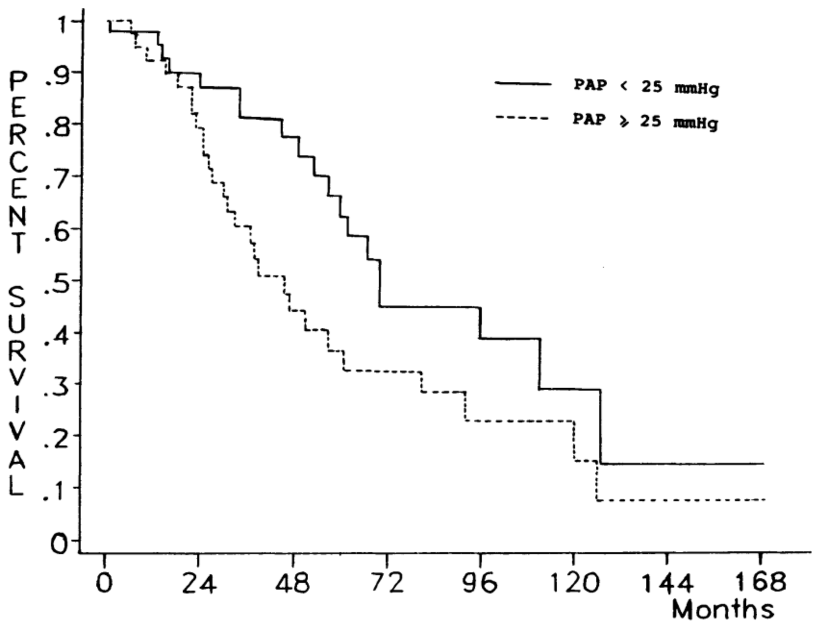

Figure 5 - Survival curve according to level of pulmonary artery pressure in COPD at onset of LTOT use

Reprinted from *Chest*, 107(5), Oswald-Mammosser M, Weitzenblum E, Quoix E et al. Prognostic factors in COPD patients receiving long-term oxygen therapy. Importance of pulmonary artery pressure, 1193-8, Copyright (1995), with permission from Elsevier.

1.2.3 Interstitial Lung Diseases

Interstitial lung diseases (ILD), also known as diffuse parenchymal lung diseases (DPLD), are a heterogeneous group of diseases with common functional characteristics (restrictive physiology and impaired gas exchange) and a common final pathway, eventually leading to irreversible fibrosis.[41-43]

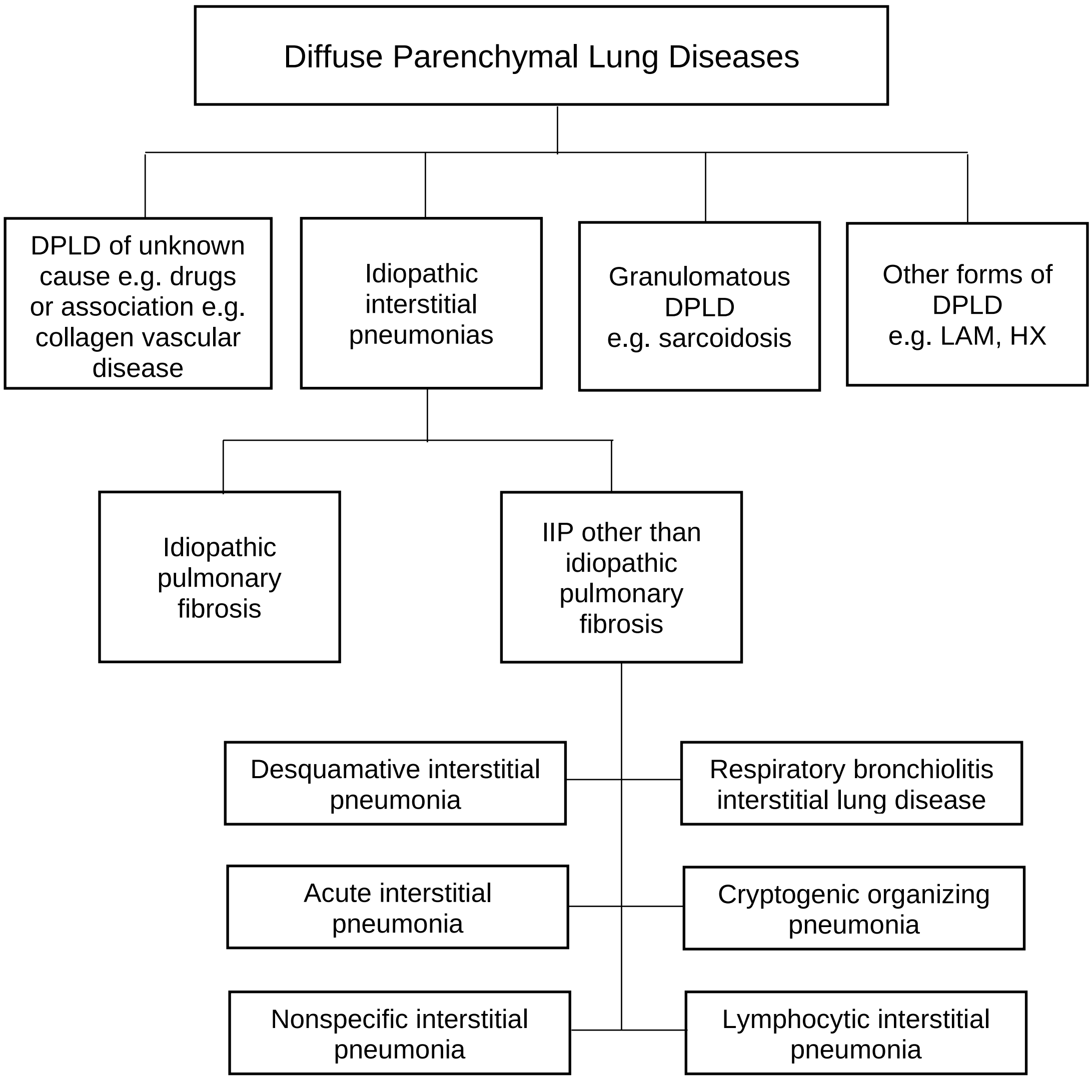

Figure 6 - Group of diffuse parenchymal lung diseases[41]

ILD result from damage to the lung parenchyma by varying patterns of inflammation and fibrosis. Some categories of ILD are associated with occupational or environmental exposures and/or collagen vascular disease, granulomatous lung diseases and some have unknown cause (idiopathic interstitial pneumonias).

The symptoms that are common in patients with ILD are chronic and progressive breathlessness, and chronic cough. Fine crackles and finger clubbing may be present on physical examination. High-resolution computed tomography of the thorax is an integral part of the evaluation of patients with ILD. Some patients may proceed to have surgical lung biopsy for firm clinicopathologic diagnosis which then allows the patients and the clinician to make more informed decisions about treatment.[41]

1.2.4 Pulmonary Hypertension in Interstitial Lung Diseases

The development of pulmonary hypertension in the context of ILD is increasingly a major adverse factor in the natural history of ILD.[43,44] The diagnosis of PH may be missed in ILD patients because the clinical symptoms of PH (dyspnoea, fatigue and exercise limitation) are also symptoms characteristics of ILD. Pulmonary hypertension in ILD (PH-ILD) is usually diagnosed when clinical signs of right heart failure develop.[45] The degree of PH-ILD is most often mild-to-moderate (resting MPAP $\geqslant$ 25 mm Hg, but < 35 mm Hg).[46]

Prevalence

The prevalence of PH in various ILD varies widely according to diagnosis and severity of the lung impairment. The most common ILD associated with PH are sarcoidosis, connective tissue diseases (especially systemic sclerosis and rheumatoid arthritis) and idiopathic pulmonary fibrosis (IPF). In the pre-transplant setting, PH is detected by RHC in 28-46% of patients suffering from fibrotic lung diseases (with majority from IPF).[12,47] Higher percentages of PH are found in advanced IPF (30% to 50%) and end-stage IPF cases (> 60%).[8,48]

Pathophysiology

The pathophysiological mechanism underlying the development of PH-ILD is poorly understood and is still the subject of investigation.[24] Similar to PH-COPD, hypoxic pulmonary vasoconstriction and vascular remodelling are considered the major culprits for increased peripheral vascular resistance in

PH-ILD.[24,44,45,49,50] However, other disease-specific mechanisms are likely also involved in the development of PH. Additional mechanisms underlying PH-ILD include the obliteration of the vascular bed by progressive parenchymal fibrosis, inflammatory response within the pulmonary vasculature specific to underlying disease, endothelial dysfunction (with reduced levels of nitric oxide and prostacyclins, and increased levels of endothelin-1 and thromboxanes), oxidative stress and growth factors.[24,44,50-52] In addition, low operating lung volumes in ILD can also raise the pulmonary vascular resistance as terminal airways tend to collapse in low lung volumes, thereby aggravating alveolar hypoxia and propagating hypoxic pulmonary vasoconstriction.[53,54] The complexity of the pathogenesis of PH-ILD is illustrated in figure below.

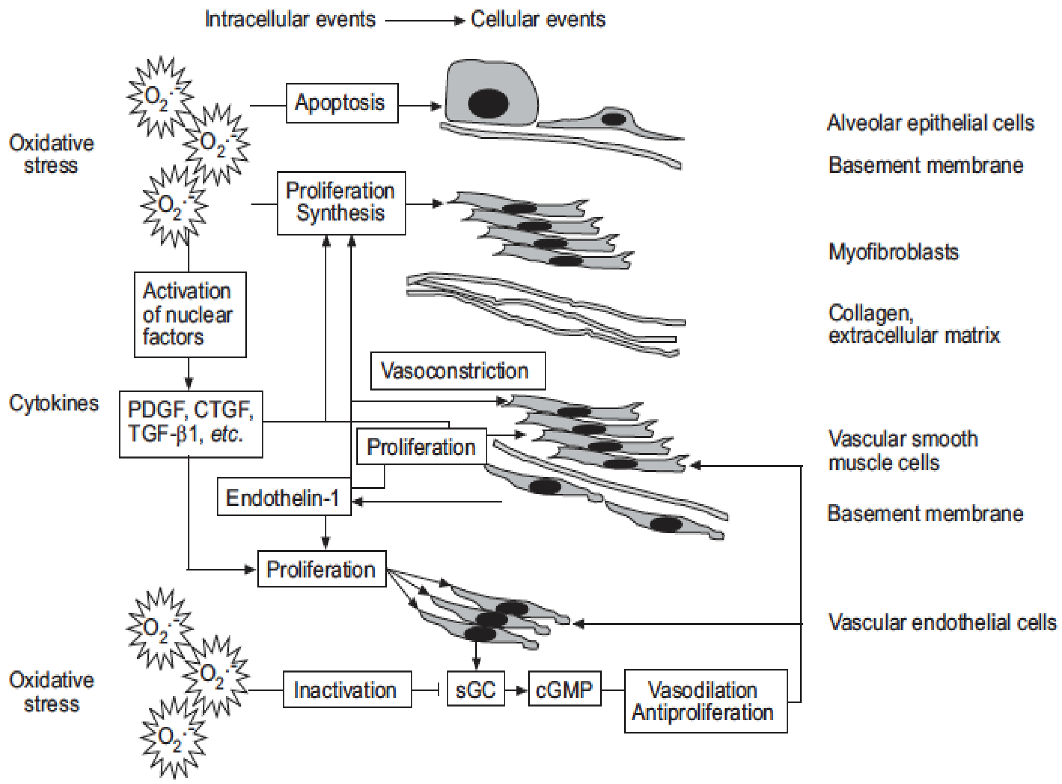

Figure 7 - Potential common pathophysiological mechanisms of parenchymal and vascular remodelling in ILD and PH[43]

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In idiopathic pulmonary fibrosis, epithelial damage has been shown to promote pulmonary vascular remodelling via release of mediators (TGF-α and pulmonary vascular smooth muscle growth factor)[55]. Moreover, elevated concentration of endothelin-1, which is a potent pulmonary vasoconstrictor, has been detected in patients with IPF with and without associated PH.[56,57]

Prognostic implications

Pulmonary hypertension has been associated with reduced survival in patients with ILD (as illustrated in *Figure 8*).[12,24,43] Hamada *et al.*[58] have also demonstrated that the 5-year survival is reduced in patients with PH (16.7%) as compared to patients without (62.2%).

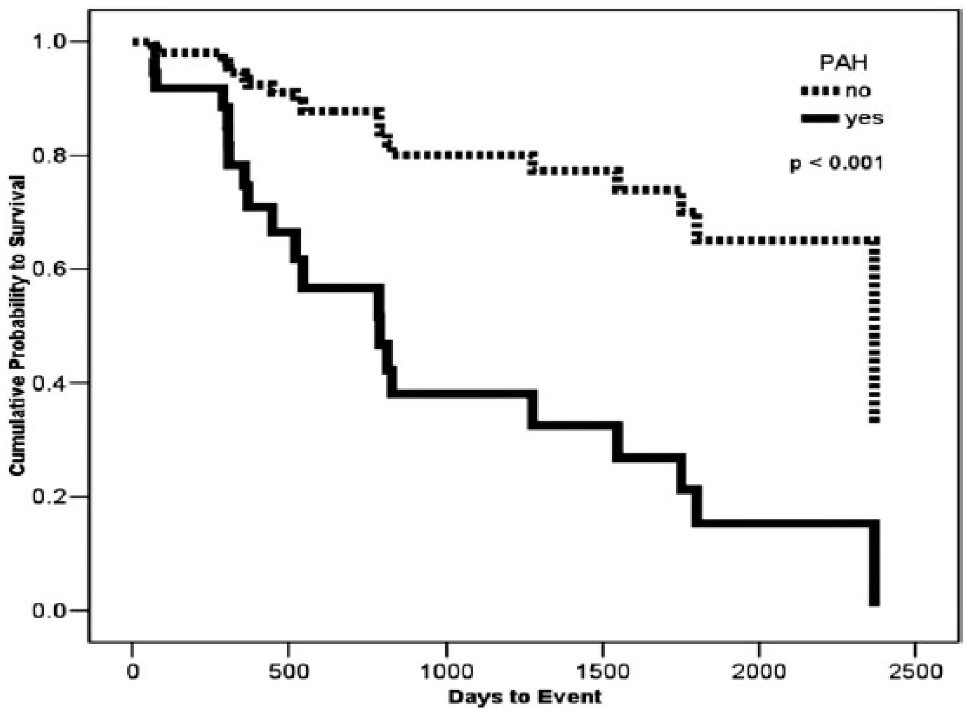

Figure 8 - PAH as a predictor of survival in patients with IPF[12]

Reprinted from *Chest*, 129(3), Lettieri CJ, Nathan SD, Barnett SD, Ahmad S, Shorr AF. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis, 746-52, Copyright (2006), with permission from Elsevier.

The presence of PH in ILD is related to decreased diffusion capacity and the need for oxygen supplementation. Relative to measures of pulmonary function and hypoxia, altered pulmonary haemodynamics have a greater impact on exercise limitation (as measured by 6-minute walk distance) in IPF patients with PH compared with IPF patients without PH who have equally severe restrictive lung physiology.[48,59]

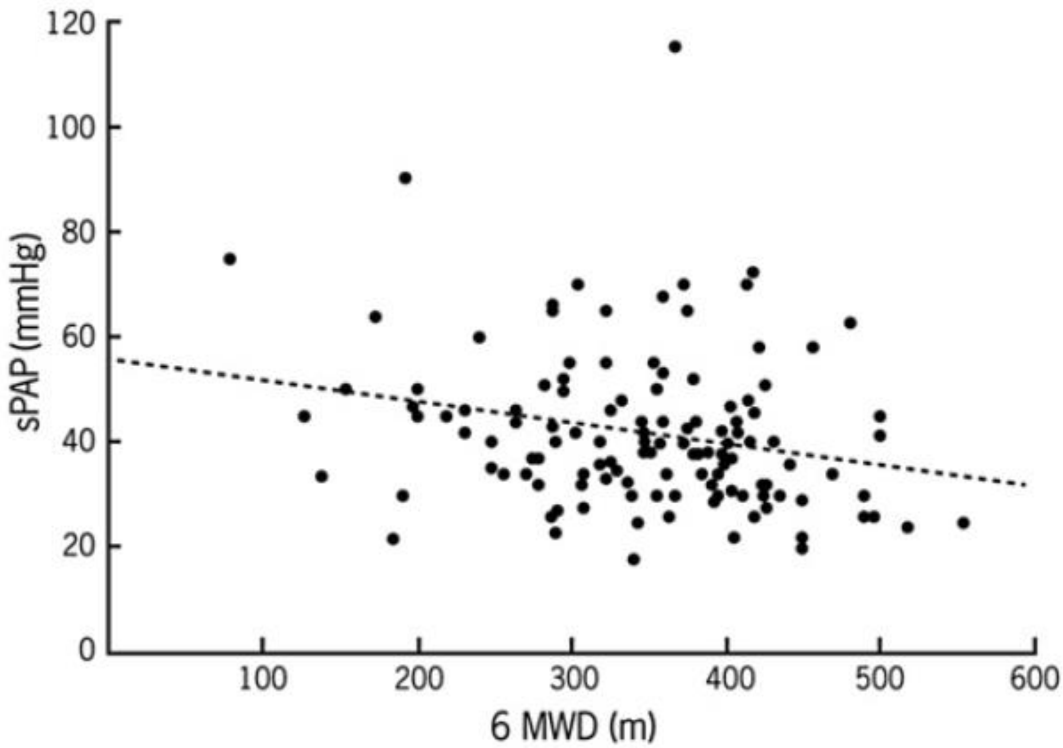

Figure 9 - Correlation of 6MWD with systolic pulmonary artery pressure

Reprinted from *Respiratory Medicine*, 106(11), Minai OA, Santacruz JF, Alster JM, Budev MM, McCarthy K. Impact of pulmonary hemodynamics on 6-min walk test in idiopathic pulmonary fibrosis, 1613-21, Copyright (2012), with permission from Elsevier.

1.2.5 Similarities in pathophysiological mechanism of development of PH in COPD and ILD

The possible mechanisms for the development of pulmonary hypertension in COPD and ILD have been described in detail in sections 1.2.2 and 1.2.4 respectively. Although there are some differences in the pathophysiological mechanisms, there is a common theme of chronic hypoxia and increased oxidative stress involved in both chronic lung diseases. Increased oxidative stress has also a role in the underlying mechanism of ventricular hypertrophy. This is discussed in more details in section 1.4.4.

1.2.6 Therapeutic options

The mainstay management of pulmonary hypertension associated with chronic lung diseases (PH-CLD) is the optimising of the treatment for the underlying lung disease. Currently, there is no specific therapy recommended for PH due to lung disease.[1] The published experience with targeted PAH drug therapy is limited and so far there is no adequate evidence to suggest that PAH drugs result in improved symptoms or outcomes in patients with PH due to lung disease. Hence the use of drugs approved for PAH (group 1)[2] is not recommended for patients with PH due to lung disease (group 3).

Long-term oxygen therapy (LTOT)

The NOTT trial and the MRC trial have shown that long-term oxygen therapy was associated with improved mortality in patients with COPD.[60,61] Both trials and the study by Weitzenblum *et al.* (1985) demonstrated that the pulmonary arterial pressure rarely returns to normal values.[44,61] The NOTT trial showed a slight decrease in pulmonary vascular resistance and Weitzenblum *et al.* study showed a slight decrease in pulmonary arterial pressure in a high percentage of patients with severe COPD. In contrast, the MRC trial showed no change in pulmonary arterial pressure.

In summary, there is no evidence to explain why oxygen therapy improved mortality in these patients. It is thought that perhaps oxygen therapy nullifies some of the effects of chronic hypoxia in these chronic lung diseases but so far, the mechanism by which this occurs is still unclear.

Nitric oxide

Endogenous nitric oxide (NO) facilitates smooth muscle relaxation and decrease pulmonary vascular resistance. Inhaled NO can worsen gas exchange in patients with COPD because of impaired hypoxic regulation of the matching between ventilation and perfusion.[62] Pulsed inhalation of mixed nitric oxide and oxygen, when compared to LTOT in stable COPD patients with PH, improved MPAP and PVR.[24] Nitric oxide is not a recommended treatment for PH-CLD.

Phosphodiesterase-5 Inhibitors

Inhibiting the enzyme phosphodiesterase-5 (PDE-5) increases the concentration of cyclic guanosine monophosphate (cGMP) in pulmonary arterial smooth muscle cells, thereby increasing NO stimulation, resulting in vasodilation.[24]

Despite improving pulmonary haemodynamics at rest and during exercise[63], sildenafil did not improve exercise tolerance in patients with PH-COPD undergoing pulmonary rehabilitation[64]. The main drawback of PDE-5 inhibitor therapies is the significant worsening impairment of arterial oxygenation due to inhibition of hypoxic vasoconstriction.[63] Sildenafil did not have a beneficial effect on exercise capacity in patients with COPD and emphysema without pulmonary hypertension. Goudie *et al* (2014)[65] demonstrated that using tadalafil did not improve exercise capacity or quality of life despite exerting pulmonary vasodilation in patients with PH-COPD.

There were mixed results in studies with patients with PH-ILD. Sildenafil caused preferential pulmonary vasodilation and improved gas exchange in patients with severe lung fibrosis and secondary PH in a small open-label study of 16 patients.[66] Sildenafil also preserved 6MWD and improved the St George's Respiratory Questionnaire (SGRQ) score compared with placebo in the subgroup of 22 patients with right ventricular dysfunction in a controlled trial of 180 patients with advanced IPF.[67] The results from Collard *et al* (2007) small open-label study of 14 IPF patients with PH were promising. They demonstrated improvement in 6-minute walk distance (6MWD) by sildenafil.[68] However, STEP-IPF (Sildenafil Trial of Exercise Performance in Idiopathic Pulmonary Fibrosis) trial which followed Collard *et al.* trial[68] and enrolled 180 patients, demonstrated that sildenafil did not improve 6MWD in patients with advanced IPF.[69]

Overall, there is still not enough evidence to support the use of phosphodiesterase-5 inhibitors as a treatment option for PH-CLD.

Endothelin Receptor Antagonists

Endothelin-1 (ET) is secreted from endothelial cells and is a powerful vasoconstrictor inducing calcium release from the sarcoplasmic reticulum and causing smooth muscle contraction.[24] Bosentan is a non-selective endothelin receptor antagonist while ambrisentan and macitentan are selective endothelin receptor A antagonists.

A small trial of 16 patients with PH-COPD reported improved exercise capacity upon treatment with bosentan.[70] This was in contrast with the results of another small randomised clinical trial (RCT) of 30 patients with PH-COPD which showed that bosentan did not improve exercise capacity, lung function and pulmonary arterial pressure but instead caused significant deterioration in hypoxaemia and quality of life.[71]

Negative RCT results have already been reported in IPF for all three ET receptor antagonists. B-PHIT study[72] demonstrated that bosentan did not improve invasive pulmonary haemodynamics, functional capacity or symptoms over 16 weeks in patients with PH associated with IPF. Similarly, BUILD-3 study[73] reported that bosentan did not delay worsening of IPF or death and had no treatment effects on health-related quality of life or dyspnea. ARTEMIS-IPF (placebo-controlled study to evaluate safety and effectiveness of ambrisentan in idiopathic pulmonary fibrosis) was terminated because of an increased rate of disease progression and respiratory hospitalisation.[74] This led to ambrisentan being contraindicated in IPF patients, regardless of the presence of pulmonary hypertension. It has been observed in the MUSIC (Macitentan USe in an Idiopathic pulmonary fibrosis Clinical study) trial that macitentan had no effects on pulmonary function tests or time to disease worsening or death for patients with IPF.[75]

Endothelin receptor antagonists are not a recommended treatment for PH-CLD.

Prostacyclin

Prostacyclin is a vasodilator secreted from endothelial cells. Different forms of prostacyclins have been used in the treatment of patients with PH with varying success.[24]

Cicletanine, a furopyridine-derative drug (shown to enhance production of endogeneous prostacycylin) has shown to significant decrease mean pulmonary artery pressure and total pulmonary resistance after three and twelve months of treatment by inducing effective pulmonary vasodilatation in PH-COPD but no significant change in oxygenation.[76]

Inhaled prostacyclin and iloprost caused marked pulmonary vasodilatation with maintenance of gas exchange and systemic arterial pressure but long-term therapy resulted in unequivocal clinical improvement.[77]

12-week treatment with parenteral treprostinil improved right heart haemodynamics and echocardiographic parameters in a small group of patients with pulmonary fibrosis with severe pulmonary hypertension (MPAP $\geqslant$35 mm Hg).[78] Sufficient evidence is lacking on the long-term use of vasodilators such as inhaled prostanoids that may preferentially access the better ventilated/oxygenated areas of the fibrotic lung due to their advantageous mode of distribution.[77]

With these mixed results, prostacyclins have not been recommended in the treatment for PH due to lung disease.

Calcium Channel Blockers

The treatment with conventional vasodilators such as calcium channel blockers is not recommended because they may impair gas exchange due to the inhibition of hypoxic pulmonary vasoconstriction[62,79] and because of their lack of efficacy after long-term use.[8,80] Nifedipine dilates pulmonary vessels constricted by hypoxia, without deleterious effects on arterial oxygenation in patients with chronic airflow obstruction and acute respiratory failure.[79]

Riociguat

Riociguat is a stimulator of soluble guanylate cyclase, a molecule that binds to NO and stimulates cyclic guanosine monophosphate.

The pilot study by Ghofrani *et al.*[81] demonstrated that a single dose of riociguat significantly improved MPAP and PVR but no relevant change in lung function or gas exchange. In an open-label uncontrolled phase II trial of 22 patients with PH-ILD, oral riociguat decreased pulmonary and systemic vascular resistance, improved cardiac output and PVR but not MPAP after 12 weeks of treatment.[82] However, a recent randomised controlled trial (www.ClinicalTrials.gov: NCT02138825) of riociguat versus placebo in pulmonary hypertension due to IPF was discontinued prematurely due to increased rates of death and serious adverse effects in the riociguat study arm.

Riociguat is not used in clinical practice to treat PH-CLD because of inadequate evidence.

1.3 Right ventricle

The right ventricle (RV) is a thin-walled, compliant and low-pressure chamber. Compared to the left ventricle (LV), the RV is thinner and has a different shape, which reflects the low pressure in the pulmonary circulation and allows quick adaptation to changes in preload.[83,84] The right coronary artery (RCA) provides blood supply to the RV free wall in both systole and diastole.[84]

The primary role of the RV is to deliver all the blood it receives into the pulmonary circulation on a beat-to-beat basis without causing the right atrial pressure to rise.[85] The RV pumps the same stroke volume as the LV with approximately 25% of the stroke work.[83,84] The septum and the free wall contribute approximately equally to the right ventricular function. The RV contraction occurs by longitudinal shortening and occurs in a peristaltic fashion starting with inflow tract contraction and proceeding to RV mid-wall, then the RV outflow tract contraction.[85]

The blood the RV receives is called the venous return and in steady state, it equals the cardiac output.[85] The rate of venous return is a function of the pressure gradient for the flow back to the heart from the periphery and its resistance.

The RV afterload is determined by the wall tension of the RV. Under normal conditions, the RV afterload is highly dependent on the distribution of blood flow in the lung, the degree of hyperinflation or increased alveolar pressure that may be present, and active increases in pulmonary vasomotor tone as may occur with inflammation and alveolar hypoxia.[85]

1.3.1 Right ventricle and pulmonary hypertension

The adaptive process associated with chronic pulmonary hypertension have been extensively described (*Figure 10*). Chronic pulmonary hypertension induces right ventricular hypertrophy (RVH). Early in the process, the RVH initially develops in the pulmonary outflow tract because this is the last region of the contracting RV to see the increased pressures and this will have the highest wall stress. The RV adaptation to pulmonary hypertension proceeds from minimal outflow tract hypertrophy with normal right atrial pressure, to generalised RV hypertrophy with sustained elevated right atrial pressure, to end-stage dilated cardiomyopathy identical to end-stage LV failure.[85]

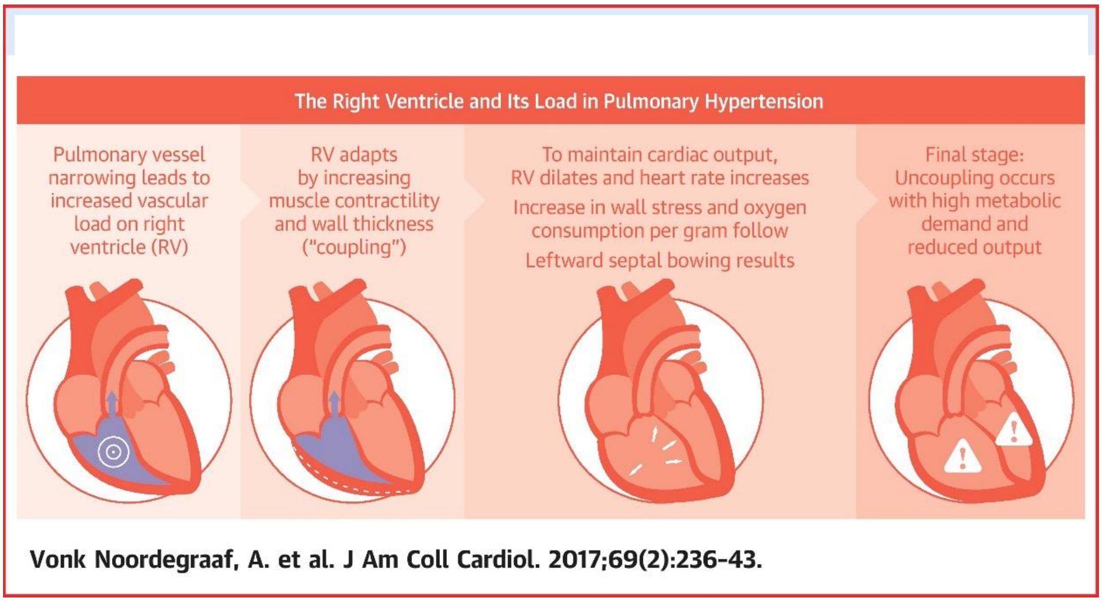

Figure 10 - The right ventricle and its load in pulmonary hypertension[86]

The challenge for the right ventricle in pulmonary hypertension is to remain coupled to its load.[86] In the early phase of the disease, the coupling of the RV and its load is maintained by a 4- to 5-fold increase in contractility of RV. The important mechanism to achieve this includes muscle hypertrophy leading to an increase in wall thickness, as well as changes in muscle properties per se. By thickening the wall, the ventricle tends to normalise the wall stress. However, as the disease advances, the hypertrophic process is halted and stroke volume (SV) decreases. The only mechanism to preserve SV is then right ventricular dilation. In an attempt to maintain cardiac output (CO) with the decreasing SV, the heart rate increases. Thus, RV uncoupling will occur in advanced stages of disease and during exercise. Compared with LV adaptation until heart failure, the RV can remain coupled for the large increase in load.

The ventricular response to load also affects the diastolic function.[85] The hypertrophy itself makes the ventricle stiffer, but changes in muscle properties add to this effect. The right atrial (RA) pressure and volume can be considered as surrogate measures of RV stiffness but those measures are load dependent. Increasing diastolic stiffness is associated with poor prognosis.[86]

When the RV hypertrophies and dilates, this may result in RV ischaemia because the RCA is unable to provide adequate blood flow to the increased RV muscle mass, which is caused in part by the reduced RCA to RV cavity pressure gradient in both systole and diastole.[85,87,88] As the pulmonary hypertension progresses, there is increased oxygen consumption because of altered metabolism, mitochondrial dysfunction and inadequate contraction patterns.[86]

Although the initial RVH in response to pressure overload may be adaptive, it is also the seemingly initial step in remodelling process that is ultimately damaging, perhaps irreversibly so.

1.3.2 Right ventricle in chronic obstructive pulmonary disease

The classic view of the development of right ventricular hypertrophy in patients with chronic obstructive pulmonary disease (COPD) is that a reduction of pulmonary vascular bed and hypoxia-induced pulmonary vasoconstriction increase pulmonary vascular resistance, resulting in pulmonary hypertension.

The pathophysiology of the RVH in COPD is more complex than simply pulmonary hypertension increasing RV afterload and RV mass. This is exemplified by presence of RVH in patients with COPD before pulmonary vascular abnormalities.[89] Vonk-Noordegraaf *et al*[89] also showed that the RVH was accompanied with decreased right ventricular end-diastolic volume (RVEDV) with preserved right ventricular systolic function. Studies of autopsy in COPD have demonstrated anatomic evidence of right ventricular (RV) hypertrophy in two-thirds of patients with chronic bronchitis[90] and one-third of patients with emphysema[25]. A possible explanation is that these cardiac changes without pulmonary vascular abnormalities are due to intermittent increases in pulmonary artery pressure (PAP) that occur during exercise and/or sleep. Another explanation is that oxidative stress directly causes ventricular hypertrophy.[91]

Lung hyperinflation is commonly seen in patients with COPD. This is the result of loss of elastic recoil combined with expiratory flow limitation, promoting increased expiratory lung volume (or air trapping) and intrinsic positive end-expiratory pressure.[26] Hyperinflation also decreases the venous return, thereby further reducing the RV filling.

1.3.3 Interstitial lung diseases

Interstitial lung diseases are characterised by a diminished lung volume due to changes in the lung parenchyma. Similar to COPD, right ventricular hypertrophy in ILD is thought to be caused by pulmonary hypertension, which is the result of raised pulmonary vascular resistance due to hypoxic pulmonary vasoconstriction and vascular remodelling causing increased pulmonary vascular resistance. Additionally, it has been postulated that the stiff lung parenchyma may compromise the function of the right heart.[92] The right ventricular diastolic filling is restricted by the anatomical restriction of the pleura and thorax, contributing to cardiac dysfunction.[44]

1.3.4 Right ventricular failure

The initial adaptive response of myocardial hypertrophy is followed by progressive contractile dysfunction. The right ventricle then dilates to allow for compensatory preload and to maintain stroke volume despite reduced fractional shortening.[84] At some point, the RV is unable to adapt further to increased RV afterload and right ventricular failure (RVF) will occur.[93]

However, RVF can be reversed.[94] Gorter *et al.*[95] have observed normalisation of biventricular morphology and function, emphasizing the reversibility of severe RV dysfunction, within several months after lung transplantation in patients with pulmonary arterial hypertension. The RV remodelling (RV hypertrophy, RV dilation and LV septal bowing) due to chronic thromboembolic pulmonary hypertension has also shown to be reversed after pulmonary endarterectomy.[96]

1.3.5 Left ventricular function

Under normal conditions, the impact of the right ventricle on the left ventricular function is negligible. In pressure overloaded RV, the ventricular interdependency leads to LV dysfunction. This is caused by leftward septal bowing, hampering filling of the LV (parallel interaction) and by decreased filling of the LV due to lowered RV stroke volume.[86]

1.4 Oxidative stress

Oxidative stress (OS) arises as a result of the endogenous antioxidant defences being overwhelmed by the presence of reactive oxygen species.[97] The balance between ROS production and antioxidant defences determines the degree of oxidative stress. Many environmental stimuli (for examples: cytokines, ultraviolet radiation, chemotherapeutic agents, hyperthermia and growth factors) generate high levels of ROS that can perturb the normal redox balance and shift cells into a state of OS.

1.4.1 Reactive oxygen species

Reactive oxygen species (ROS) are radicals derived from oxygen. The sequential reduction of molecular oxygen (i.e sequential addition of electrons) leads to the formation of a group of reactive oxygen species *(Figure 11)*

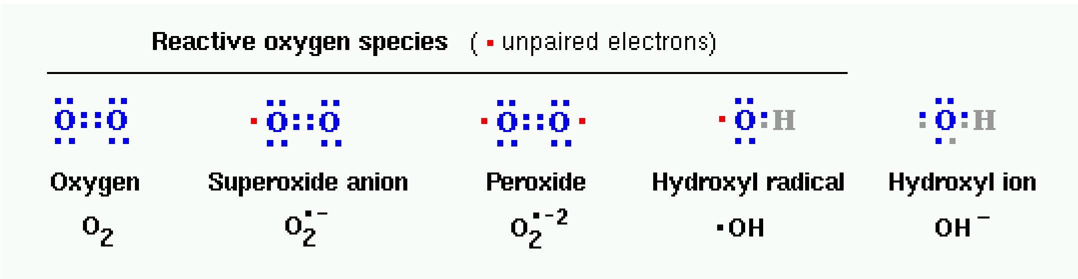

Figure 11 - Reactive oxygen species

The generation of reactive oxygen species is essential to life and to maintain homeostasis.[98] ROS are generated constantly in the mitochondria as part of normal aerobic life. Under normal metabolic, complex III (ubiquinonecytochrome c reductase) is the main site for ROS production in the electron transport chain, in the mitochondria (*Figure 12*).

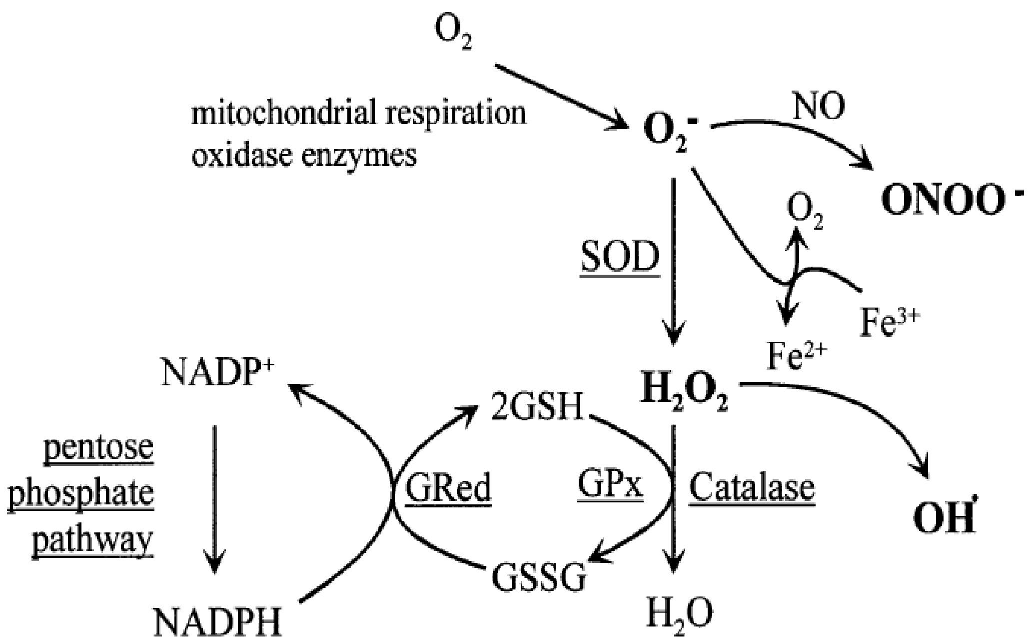

Figure 12 - Basic pathway for generation of ROS[99]

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ROS are formed as necessary intermediates in a variety of enzyme reactions. For example: ROS generation by phagocytic cells constitutes an essential host defence mechanism necessary to fight infection.[98]

However, ROS can be toxic to cells as they possess an unpaired electron, which makes them highly reactive and thereby able to damage all macromolecules, including lipids, proteins and nucleic acids. The mechanism of ROS cytotoxicity is attributed to peroxidation of membrane lipids, DNA damage and protein oxidation, which impair mitochondrial function and lead to apoptosis.[100] ROS have proinflammatory activity because they regulate endothelial functions, by both increasing the permeability of vascular lining and modulating the arteriolar tone.[100]

The burden of ROS production is largely counteracted by an intricate antioxidant defence system that includes the enzymatic scavengers such as superoxide dismutases (SOD), catalase, thioredoxin (TRX) and glutathione peroxidase (GPX). Other non-enzymatic, low molecular mass molecules scavenging ROS include ascorbic acid (vitamin C), pyruvate, flavonoids, carotenoids and most importantly, glutathione.

The xanthine oxidoreductase system, which is one of the enzymatic process involved in the production of reactive oxygen species, is being looked at in more detailed in the next section.

1.4.2 Xanthine oxidoreductase

Xanthine oxidoreductase (XOR) is the rate-limiting enzyme in the last two steps of purine catabolism in man.[100,101] XOR was first identified in milk in 1902 and is a member of the molybdenum hydroxylase flavoprotein family. [102,103]

XOR exists in two interconvertible forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO). Both forms of the enzyme carry out similar reactions of purine catabolism but the mechanisms of action are different. The conversion may occur either irreversibly by limited proteolysis or reversibly by chemical or enzymatic oxidation of XDH thiol groups.[102]

Gene, expression and regulation

The gene encoding XOR is located on the short arm of chromosome 2 and comprises of 36 exons. XOR activity is expressed at very low levels and a variety of factors upregulate transcription: hypoxia [104-106], lipopolysaccharide [107,108], interferon γ [109,110], interleukin-1 [109,110], interleukin-6 [111], tumour necrosis factor α [109], dexamethasone, cortisol and prolactin.[100,103]

Hyperoxia has previously been shown to decrease XOR activity in cell culture and rat lungs [112,113], while hypoxia was shown to induce a gradual increase in XOR activity in endothelial cells [105,112]. The mechanism by which oxygen tension affects XOR expression appears to be complex and is not very well understood at this time.[102]

Distribution

XOR is widely distributed throughout various organs including the liver, gut, lung, kidney, heart, brain and plasma.[114] Based on its levels of messenger RNA and protein expression, the highest activity levels of XOR are present in liver, intestine, kidney and lactating mammary gland epithelial cells and in vascular endothelial cells.[100]

XOR has been detected not only in the cytoplasm of cells but also on the outer surface of the endothelial cell plasma membrane.[102,103,115] XOR can be released into the systemic circulation from the liver and intestine during reperfusion after ischaemia.[102]

Physiological functions

XOR is the terminal enzyme of purine catabolism in man, catalysing the hydroxylation of hypoxanthine to xanthine and of xanthine to urate (*Figure 13*).[101]

Both XDH and XO catalyse the oxidation of hypoxanthine to xanthine and xanthine to uric acid but their mechanisms of action are different. XDH reduces NAD^+ by a direct two-electron reduction while XO reduces molecular oxygen by a single electron.[102]

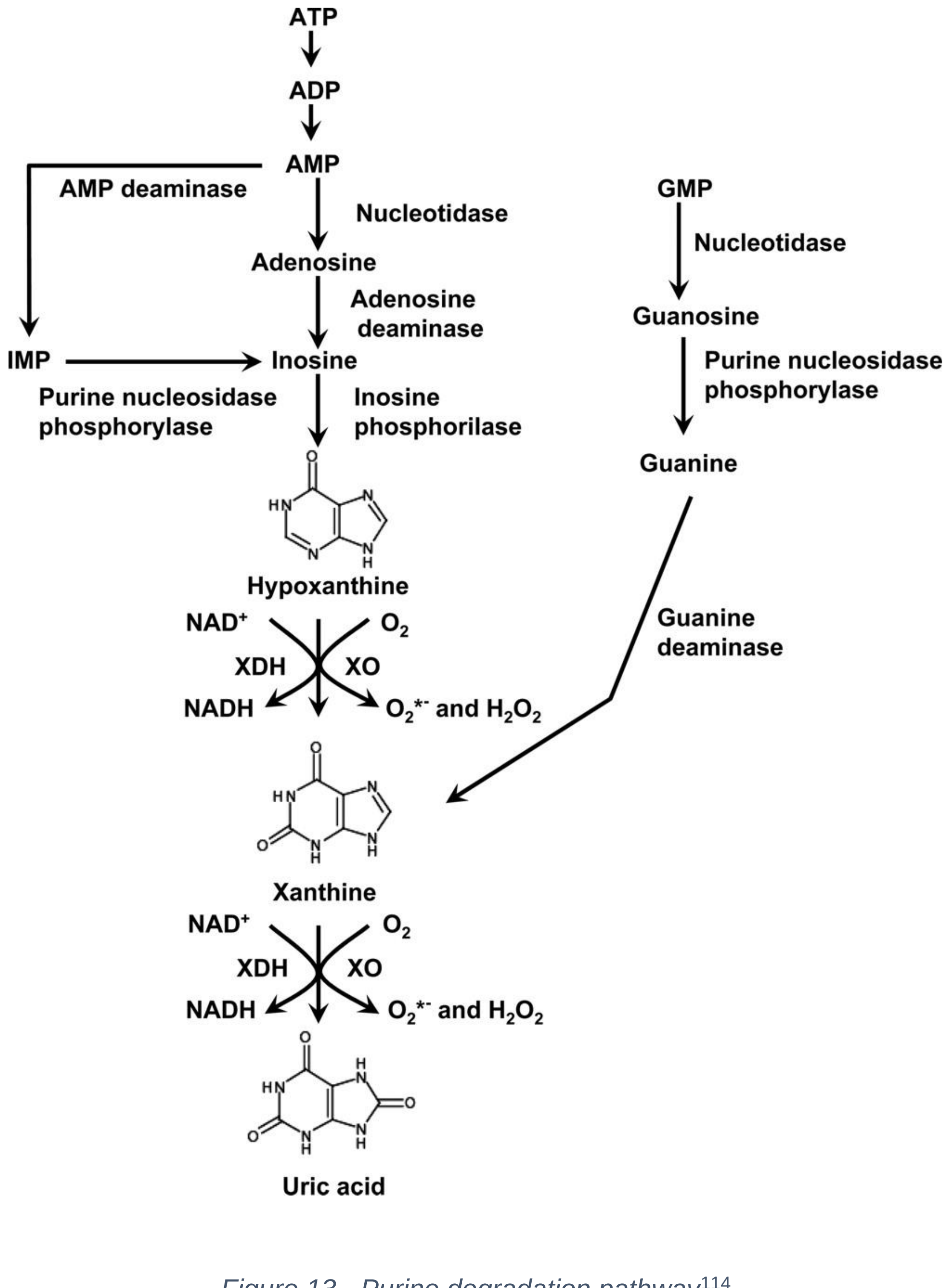

Figure 13 - Purine degradation pathway[114]

XDH requires NAD^+ as an electron acceptor to reduce hypoxanthine to xanthine (or xanthine to urate), thereby generating the stable reaction product NADH. XO is unable to use NAD^+ as an electron acceptor, requiring instead the reduction of molecular oxygen for this purine oxidation, thereby generating the highly reactive superoxide free radical (O_2^-) and hydrogen peroxide (H_2O_2).[116]

Pathophysiology role

It is well established that XOR can act as a source of superoxide and hydrogen peroxide, which could exert protective (e.g bactericidal) or destructive effects. H_2O_2 generated by XO are dependent upon oxygen (O_2) tension, pH and purine concentration. Under relatively physiologic conditions (21% O_2 and pH 7.0) XO catalyzes the reduction of O_2 to H_2O_2 and $O_2^{\cdot -}$ at a ratio of 4:1 or ~80% H_2O_2 and ~20% $O_2^{\cdot -}$.[117]

H_2O_2 formation is further favoured when both O_2 levels and pH are reduced.[100] Kelley *et al.*[117] data confirmed an oxygen dependence of ROS formation in that lower oxygen concentrations lead to even greater H_2O_2 formation by XO. This is critical to note as hypoxia / inflammation leads to lower O_2 tensions, increased XO expression, and increased hypoxanthine / xanthine levels from ATP catabolism and thus sets the stage for enhanced vascular H_2O_2 production. H_2O_2 has the potential to have significant deleterious effects.

1.4.3 Oxidative stress in the lungs

The lungs are constantly exposed to sources of endogenous oxidative stress generated by mitochondrial respiration and inflammatory responses to bacterial and viral infections within the lungs.[97] In the lungs, the endothelial cells, neutrophils, eosinophils, alveolar macrophages and alveolar epithelial cells are all major sites of ROS generation.[118] Other sources of intracellular ROS include NADPH oxidase, the xanthine/xanthine oxidase system and the heme peroxidases.

Elevated levels of ROS have been found in COPD and these may be associated with increased inflammation, airway remodelling, autoimmunity and corticosteroid resistance.[97] Based upon a large number of studies in animal models, the three major stimuli that drive the vascular remodelling process via ROS in pulmonary hypertension are inflammation, shear stress and hypoxia.[118]

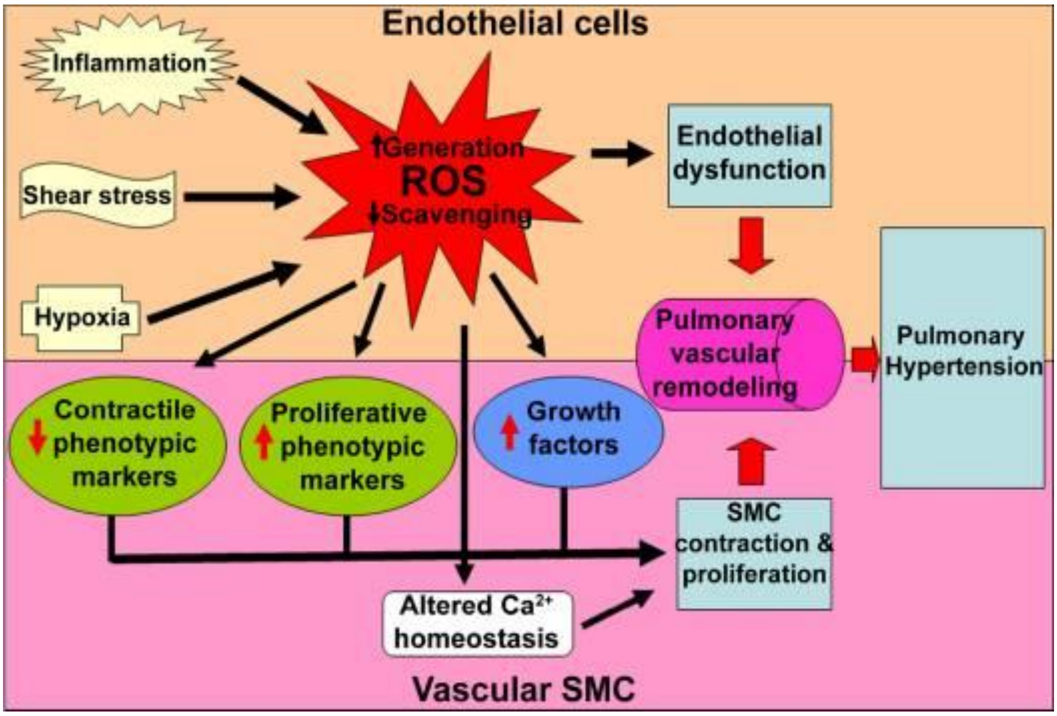

Figure 14 - Schematic view of pathology of pulmonary hypertension[118]

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In the endothelial cells, ROS promote endothelial proliferation, decrease nitric oxide (NO) and increase the release of vasoactive mediators, leading to endothelial dysfunction. In smooth muscles, OS caused by ROS induces contraction.

1.4.4 Oxidative stress in cardiac hypertrophy and remodelling

Cardiac hypertrophy occurs in the presence of chronic hypertension. It could be a direct effect from chronic overload state (as discussed in section 1.3.1) but it could also be due to a direct effect of oxidative stress on the heart.

ROS can be formed in the heart by a variety of mechanisms, including generation during oxidative phosphorylation in the mitochondria as a byproduct of normal cellular aerobic metabolism.[119] Superoxide (O_2^-) is formed intracellularly by activation of nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase or xanthine oxidase (XO), uncoupling of NO synthase (NOS) and electron transport and ‘leakage’ during oxidative phosphorylation in the mitochondria.[120] Low levels of ROS are thought to play a role in normal cardiac signalling, growth adaptations and matrix changes. Higher levels play a role in the pathophysiologic remodeling, apoptosis and chamber dysfunction (*Figure 15*).

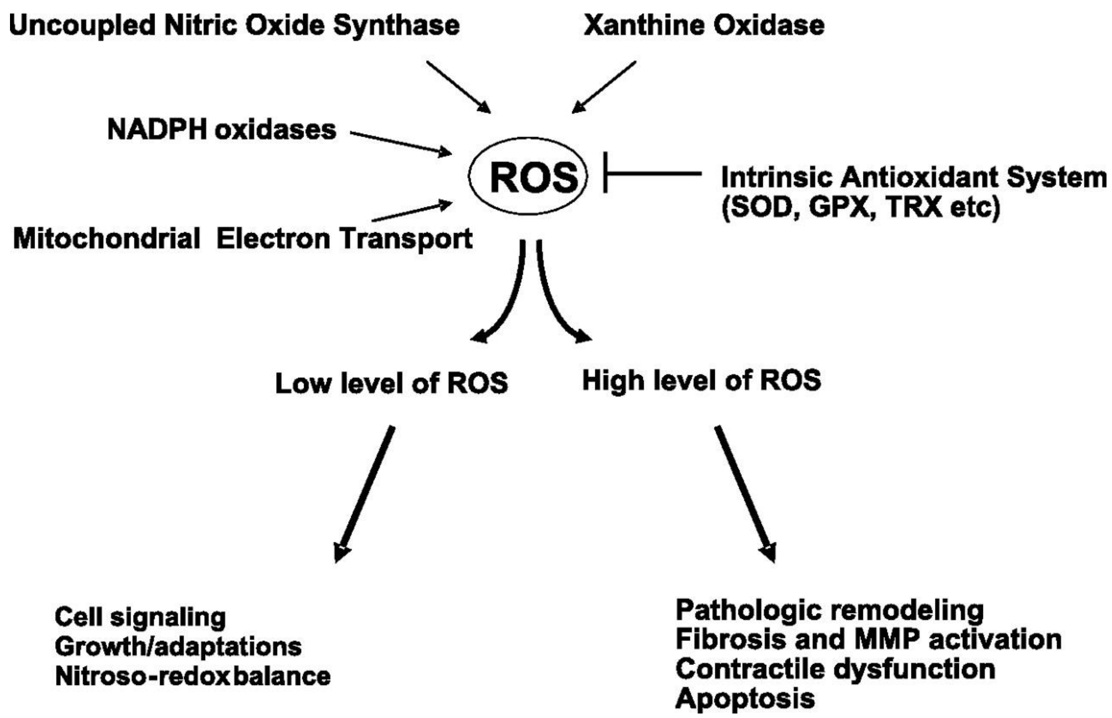

Figure 15 - General schematic of generation pathways for ROS and antioxidant systems in the heart[120].

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Under conditions of afterload stress (pulmonary hypertension or systemic hypertension), both ventricles increase ROS production.[121] Increased myocardial ROS levels might also reflect increased activity of intracellular oxidase complexes such as NADPH oxidase, xanthine oxidase or nitric oxide synthase. ROS have potent effects on the extracellular matrix, stimulating cardiac fibroblast proliferation, activating matrix metalloproteinase, effects central to fibrosis and matrix remodelling. ROS may mediate the hypertrophic response to well-recognised hypertrophic stimuli in redox sensitive pathways, such as mechanical strain, angiotensin, tumour necrosis factor-α and α-adrenergic receptor stimulation (*Figure 16*)[99]

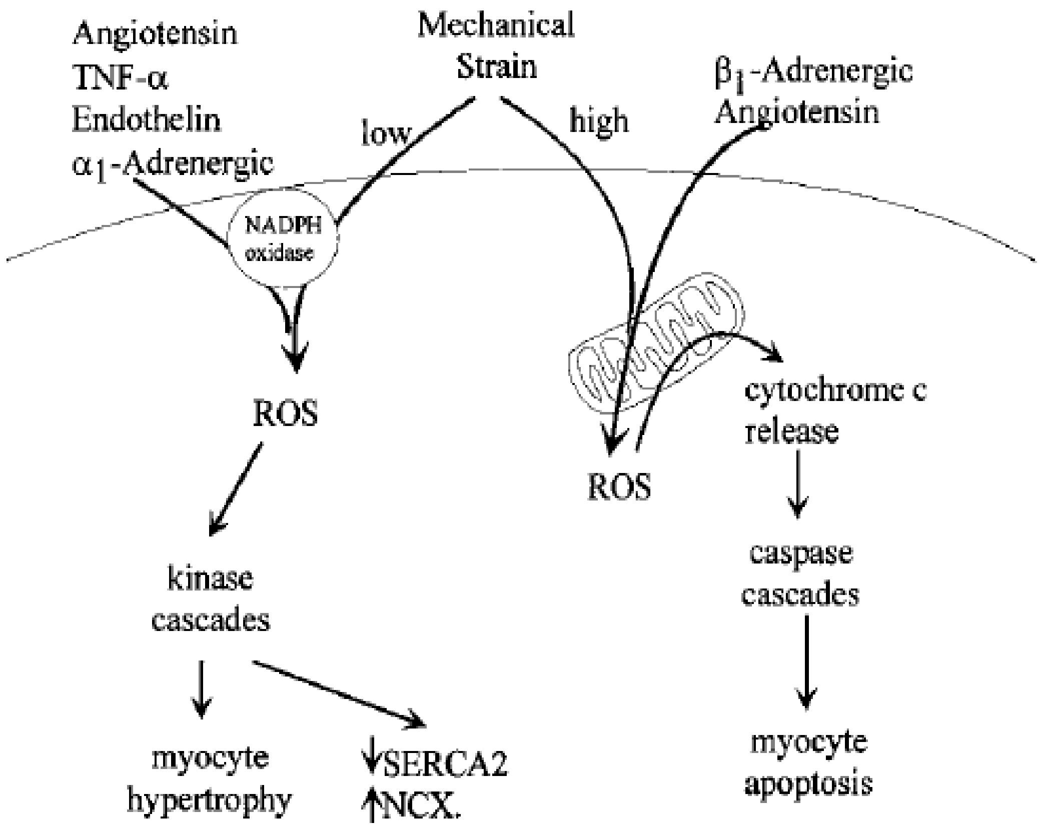

Figure 16 - ROS mediated myocyte hypertrophy[99]

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In the pulmonary hypertension-stressed right ventricle, the antioxidant enzymes superoxide dismutase and glutathione peroxidase are not activated at all in the compensate stage, predisposing the RV to ROS-induced damage at an earlier stage than in the LV.[122]

Wang *et al* (2017) experimental study in rats have demonstrated that RV failure is associated with the increased production of ROS by XO.[123]

1.5 Uric acid

Uric acid (interchangeably referred as urate) is the final product of purine nucleotides metabolism[124]. Uric acid (UA) is produced mainly in the liver and is excreted by the kidneys.

Hyperuricaemia has been associated with increased risk of adverse cardiovascular outcomes in the general population.[125] Several epidemiological studies have demonstrated a relation between serum UA levels and a wide variety of cardiovascular conditions, including hypertension, metabolic syndrome, coronary artery disease, cerebrovascular disease, vascular dementia, pre-eclampsia and chronic kidney disease.[126] This relationship is observed in frank hyperuricaemia (> 360 μmol/L in women, >420 μmol/L in men) and also in normal to high range (310 – 330 μmol/L). Wei *et al.* (2010) cohort study of 7135 patients reported that high-dose (≥ 300mg) of allopurinol was associated with better control of urate level and lower risks of both cardiovascular events and mortality.[127]

Serum and urinary UA levels are reported to be useful markers reflecting hypoxia in patients with various diseases such as obstructive sleep apnoea syndrome, chronic obstructive pulmonary disease, chronic heart failure, primary pulmonary hypertension, cyanotic congenital heart disease and Eisenmenger syndrome.[128,129] Serum uric acid levels are elevated in patients with idiopathic pulmonary arterial hypertension (IPAH). [130] Increase in serum UA levels was proportional to clinical severity of IPAH and have a strong, independent association with long-term mortality of patients with IPAH.

Uric acid and its oxidized derivatives may exert pro-oxidant activity, mainly within the cell; however, it has in vivo antioxidant activity mainly in body fluids.[100]

In summary, serum uric acid levels are elevated in hypoxic conditions such as chronic lung diseases (COPD and ILD). There is currently no evidence of any relationship between serum uric acid and pulmonary hypertension due to chronic lung diseases.

The next section will discuss about allopurinol which is licensed and used for decreasing serum uric acid,and has been shown to be an antioxidant.

1.6 Allopurinol

1.6.1 Background

Allopurinol (1,5-dihydro-4H -pyrazolo[3,4-d]pyrimidin-4-one) is a potent xanthine oxidase inhibitor that was developed in the 1950s by Gertrude B. Elion and George H. Hitchings.[114,131] The latter were awarded the 1988 Nobel Prize in Physiology and Medicine for its discovery.[114]

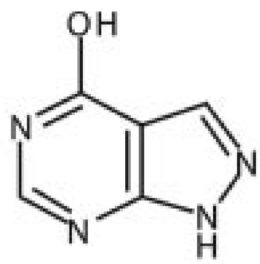

Figure 17 - Structure of allopurinol

Ellion and Hitchings have investigated several hundred purines, pyrimidines and analogues to increase the therapeutic efficacy of antineoplastic drugs such as mercaptopurine and its derivatives to discover new cancer treatments.[114,132] Since mercaptopurine is known to be converted to inert thiouric acid by the versatile enzyme xanthine oxidase, the inhibition of the enzyme seemed to be one possible way of increasing the efficiency of this cytotoxic drug.[131,133,134] Subsequently, allopurinol was found to decrease plasma concentration of urate and the occurrence of acute gout.[134]

Since its Food and Drug Administration approval in 1966, it is commonly used worldwide for prophylactic treatment of gout[134] and remains a cornerstone in the therapy of primary and secondary hyperuricaemia.[114]

1.6.2 Biochemistry

Allouprinol is an analogue of hypoxanthine. Allopurinol is a very weak acid with acid dissociation constant (pKa) of 9.4 and is therefore essentially unionised at all physiological pH values. It has low lipid solubility as indicated by its octanol:water partition coefficient of 0.28.

The active metabolite of allopurinol is oxypurinol, which is an analogue of xanthine. Oxypurinol is a somewhat stronger acid with a pKa of 7.7 and is therefore about 30% ionised in plasma (pH 7.4). Oxypurinol is more lipid soluble than allopurinol, with an octanol:water partition coefficient of 14. Oxypurinol should therefore pass more easily thorugh cell walls by passive diffusion.

Both allopurinol and oxypurinol are inhibitors of xanthine oxidoreductase (XOR), which is the enzyme essential in the oxidation of hypoxanthine to xanthine, and xanthine to urate *(section 1.4.2)*. As oxypurinol has a much longer half-life ($t_{1/2}$) than allopurinol, it is largely responsible for the hypouricaemic effect of allopurinol.

At relatively high concentrations (> 500 µM), allopurinol and oxypurinol have been shown to act as powerful scavengers of hydroxyl radicals in vitro and the possibility that the beneficial effects of these inhibitors could result from such scavenging have been examined.[101]

Allopurinol by itself cannot prevent the generation of superoxide by XOR. It is an efficient alternative substrate of XOR that must first be converted to oxypurinol, the actual inhibitor. Oxypurinol is referred to as a pseudo irreversible inhibitor that "inactivates" the enzyme. Allopurinol produces superoxide during its conversion to oxypurinol. The XO-catalyzed conversion of allopurinol to oxypurinol clearly generates superoxide during the reaction. In conclusion, allopurinol can generate superoxide during its conversion to oxypurinol as catalysed by XO. Moreover, although both allopurinol and oxypurinol are inhibitors of XOR that can block the formation of uric acid, only oxypurinol can inhibit the formation of superoxide.[135]

1.6.3 Pharmacokinetics & pharmacodynamics

Oral allopurinol is rapidly absorbed from the gastrointestinal tract. The oral bioavailability of unchanged allopurinol is quite high and estimated to be 79 $\pm$ 20%. The plasma concentration of allopurinol then declines rapidly with a mean $t_{1/2}$ of 1.2 hours.

After absorption, allopurinol is rapidly metabolised in the liver by oxidation to oxypurinol. The $t_{1/2}$ of oxypurinol is much longer (23 hours) than that of allopurinol. Oxypurinol is the metabolite that accumulates in plasma during long-term dosing with allopurinol. During the long-term treatment, the steady-state plasma concentrations of oxypurinol increase approximately linearly with the dose of allopurinol.

Following a single dose of allopurinol, the plasma concentrations of urate fall slowly, reaching a maximal decrease between about 6 and 24 hours with little recovery over the next 2 days[136,137]. In healthy subjects, the $t_{1/2}$ of urate is about 36 hours.[138] Even at the highest plasma concentrations of oxypurinol, the plasma concentrations of urate can be reduced by a maximum of 83% of the starting plasma concentrations.

Approximately 10% of the oral dose of allopurinol is excreted unchanged in urine.[131] The main route of elimination of allopurinol is via the oxidation to oxypurinol. The primary route of elimination of oxypurinol is by renal excretion. The clearance of oxypurinol is decreased in patients with renal impairment.[139,140] Allopurinol elimination is not reduced with age as it is eliminated by metabolism. However, oxypurinol elimination is reduced in the elderly because of age-dependent decline in renal function.[136]

1.6.4 Dosage

Oxypurinol concentration at steady-state increases in a linear fashion over the dosage range of 50 to 600 mg/day of allopurinol[132] *(Table 3)*. Xanthine oxidase activity decreased with increasing steady-state plasma oxypurinol concentrations

Allopurinol dose (mg/day)	Steady-state oxypurinol concentration (mg/L)
50	1.77 ± 1.59
100	2.67 ± 1.59
300	5.59 ± 1.50
600	9.56 ± 1.92
900	12.21 ± 2.13

Table 4 - Steady-state oxypurinol concentration response to allopurinol dose (adapted)[132]

Allopurinol should be introduced at low dosage (100 mg/day) to reduce the risk of adverse reactions and increased only if the serum urate response is unsatisfactory. Dosage schedules for gout are: 100-200 mg daily in mild conditions, 300-600 mg daily in moderately severe conditions, and 700-900 mg daily in severe conditions.

1.6.5 Side effects

Allopurinol is generally a well-tolerated and has been used for over 50 years for the treatment of gout. The incident of true drug reaction was found to be 1-2%.[131] The side effects are usually mild gastrointestinal or hypersensitivity reactions.[141] In a drug surveillance programme, the most common adverse effects attributed to allopurinol are skin reactions (1.8%), haematological abnormalities (0.6%), diarrhoea (0.3%) and drug fever (0.3%).[142] The reactions were unrelated to age, weight, reason for therapy, blood urea or albumin concentrations. The reactions subsided within a few days after administration of the drug was suspended.[131] Patients at higher risk of developing sensitivity reactions include those with chronic hyperuricemia, reduced renal function, advanced tophaceous gout, chronic alcoholism, and severe liver disease, and those being given thiazide diuretic therapy.[131]

The most dangerous adverse reaction of allopurinol is toxic epidermal necrolysis (TEN).[134] It is also called Stevens-Johnson syndrome or allopurinol hypersensitivity syndrome (AHS) or drug reaction with eosinophilia and systemic symptoms (DRESS syndrome).[140] The patients develop a fever, exfoliative rash, lymphadenopathy, arthralgia, eosinophilia, vasculitis, hepatitis and deterioration in renal function, and it can be fatal[141]. The EuroSCAR[143] study reported that allopurinol at daily doses of ≥ 200mg was associated with a higher risk of TEN (adjusted OR 36). The presence of HLA-B*5801 allele, which is a common allele in the Chinese population, is a very significant genetic risk factor for DRESS syndrome. All cases of the DRESS syndrome have occurred within two months of the commencement of dosage with allopurinol.[134]

1.6.6 Allopurinol as antioxidant

Several studies have looked into the potential benefits of XO inhibitors like allopurinol given the role of XOR in the production of ROS .[141]

Coronary artery bypass surgery

Giving allopurinol to patients who are scheduled to undergo coronary artery bypass surgery (CABG) has had mixed results.

Johnson *et al.* (1991) demonstrated that pre-treating these patients with allopurinol (first dose given the evening before surgery and second dose at 4 hours before scheduled surgery time) significantly improves cardiac function after surgery and is associated with lower hospital mortality rates. The hospital mortality rate was 4% in allopurinol group (n = 89) compared to 18% in placebo group (n = 80). These findings had let to a change in policy in their unit where all patients who were scheduled to undergo CABG were pre-treated with allopurinol.[144]

When allopurinol was given both pre-op (300 mg twice daily for 2 days and 600 mg on the morning of operation day) and post-op (300 mg twice daily for 2 days), patients were at a lower risk of developing cardiac complications such as myocardial infarction (none in allopurinol group vs. 17.7% in control group) and arrhythmias needing treatment (6.6% in allopurinol group vs. 33.3% in control group; $p < 0.01$). The percentage of patients requiring inotropes or intra-aortic balloon pump post-operatively was significantly lower in the allopurinol group (4.4%) compared with the control group (26.6%).[145]

In contrast, Taggart *et al.* (1994) and Coetzee *et al.* (1996) were not able to show that pre-treatment with allopurinol had a cardioprotective effect in patients with either good or impaired left ventricular function undergoing CABG. There were no significant difference in cardiac troponin T, CK-MB or myoglobin between allopurinol and control groups when measurements were done pre-op, subsequently at 1, 6, 24 and 72 hours post-op.[146] When global left ventricular function was assessed, there was no difference in the left ventricular stroke work index before or after surgery in both allopurinol and control groups.[147]

The differing nature of the cardiac operations (anaesthetic drug regime, degree of hypothermia) makes the interpretation of the results from the above four studies challenging. A possible explanation for the difference in the results is that none of the patients in Coetzee *et al.* (1996) study had beta-adrenergic blockers whereas there was no information whether patients in Johnson *et al.* (1991) and Rashid *et al.* (1991) studies had received any prior to arresting the heart. It is known that beta-adrenergic blockers are cardioprotective and hence could have been a confounding factor.

The population of Taggart *et al.* trial was smaller (n = 20) compared to Rashid *et al.* (n = 90) and Johnson *et al*. (n = 169). This could be a possible explanation for the negative result from Taggart *et al.* trial as the population size might have been too small to detect an effect.

Endothelial function

Endothelial dysfunction is a well established response to cardiovascular risk factors and precedes the development of atherosclerosis. It is characterised by reduction of the bioavailability of vasodilators and/or an increase in endothelium-derived contracting factors.[148]

Several studies have demonstrated the benefits of allopurinol on endothelial function in a variety of conditions: cigarette smoking[149], type 2 diabetes mellitus[150], chronic kidney disease[151], chronic heart failure[152,153], chronic stable angina[154] and obstructive sleep apnoea[155].

Cigarette smoking is well-known to cause endothelial dysfunction. By giving a single oral dose of 600mg of allopurinol to 14 cigarette smokers and comparing with age- and sex-matched healthy non-smoking control subjects, Guthikonda *et al.* (2003) have shown that allopurinol reversed endothelial dysfunction (assessed by forearm blood flow responses to intra-arterial administration of acetylcholine) in smokers without affecting responses in non-smokers.[149]

Butler *et al.* (2000) have suggested that free radicals could be an important and reversible cause of endothelial dysfunction in patients with type 2 diabetes mellitus. They have found that after giving 300 mg allopurinol for one month, the endothelial function, which was assessed by forearm venous-occlusion plethysmography (FVOP) with intra-arterial infusion of acetylcholine, was increased and that the level of malondialdehyde, which is an indirect measure of free radical activity, was also significantly reduced by allopurinol.[150]

Improvement in endothelial function with allopurinol was confirmed in patients with chronic kidney disease (CKD). Kao *et al.* (2011) reported that endothelial function, assessed by flow-mediated dilation (FMD) of the brachial artery, and arterial stiffness, evaluated by pulse-wave analysis (PWA), were both improved after giving 9 months of allopurinol 300 mg/day to patients with CKD.[151]

Farquharson *et al.* (2002) found that allopurinol increased forearm blood flow response to acetylcholine with a reduction in plasma malondialdehyde level in their randomised, placebo-controlled, cross-over study on 11 patients with New York Heart Association (NYHA) class II-III chronic heart failure.[152] This positive result in chronic heart failure was confirmed in two other small studies done by Doehner *et al.* (2002). The latter have demonstrated that allopurinol improved endothelial function, and the blood flow in the arms and the legs, along with a decreased allantoin level (another marker of oxygen free radical generation) in hyperuricaemic patients with chronic heart failure.[153]

High-dose allopurinol (600 mg/day) improved endothelial function (assessed by FVOP, FMD and PWA) of patients with stable chronic angina. Rajendra *et al.* (2011) also showed that vascular tissue oxidative stress was profoundly reduced.[154] Based on these two findings, the authors allude the idea that allopurinol may reduce future cardiovascular mortality in stable coronary artery disease. This idea has been taken on board by Mackenzie *et al.* (2016) who are currently recruiting for the ALL-HEART study to investigate whether allopurinol improves cardiovascular outcomes in patients with ischaemic heart disease.[156]

The severity of endothelial dysfunction correlated with the severity of sleep apnoea in El Solh *et al.* (2006) study.[155] The other study findings were that allopurinol improved endothelial dysfunction and reduced plasma malondialdehyde levels in patients with moderate-to-severe obstructive sleep apnoea, who were given 300 mg allopurinol daily for 2 weeks.

George *et al.* (2006) investigated the mechanism of action of allopurinol on endothelial function by comparing probenecid, which would only reduce urate level, versus placebo. Their study confirmed that the mechanism of improvement in endothelial function with allopurinol lies in its ability to reduce vascular oxidative stress and not in urate reduction. They also found that increasing the dose of allopurinol from 300 mg to 600 mg improved the endothelial function by 52%.[157]

Chronic heart failure

Ekelund *et al.* (1999) study has suggested that allopurinol can act as a novel inotropic agent that simultaneously decreases oxygen consumption and markedly increases myocardial mechanical efficiency in (pacing-induced) heart failure (in dogs).[158] This idea was further supported by Cappola *et al.* (2001) study which has shown that XO activity may contribute to abnormal energy metabolism in human cardiomyopathy and short-term administration of allopurinol improves myocardial efficiency.[159] Hirsch *et al.* (2012) further sustained the concept that allopurinol enhances the energetic profile of the failing human heart by increasing adenosine triphosphate (ATP) availability and increasing the amount of energy released with each ATP molecule hydrolysed.[160] Opie (2012) proposed the potential sites of action of allopurinol for heart failure *(Figure 18)*.[161]

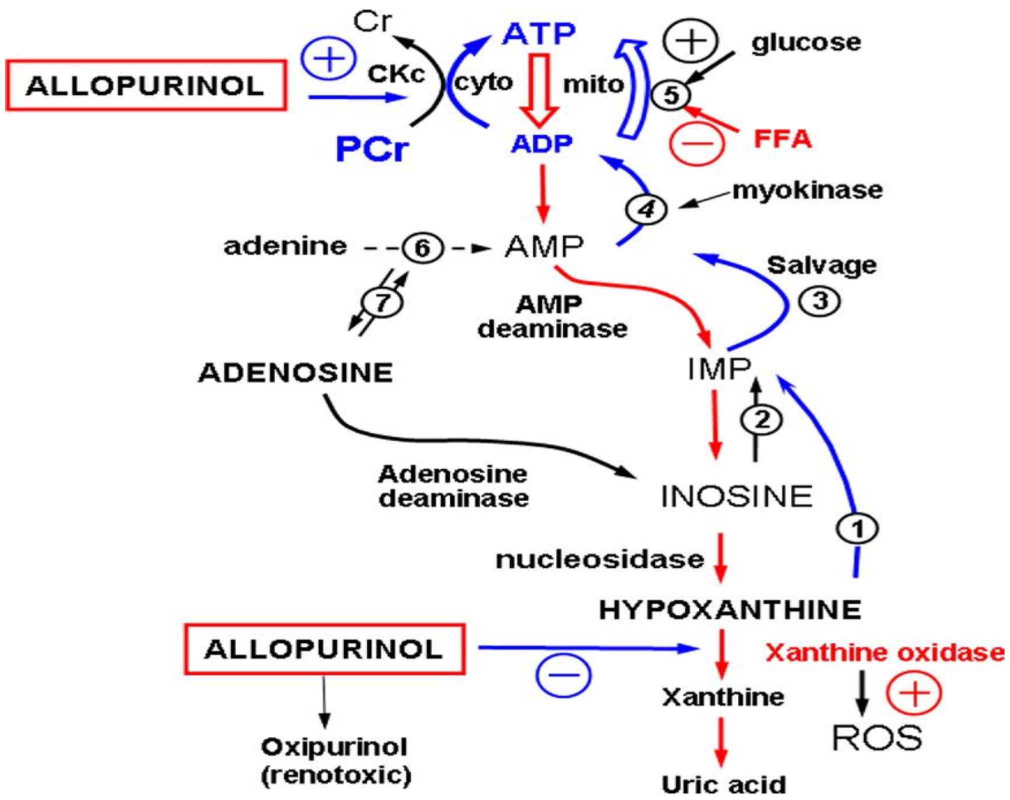

Figure 18 - Proposed site of action of allopurinol in heart failure[161]

Inhibition of xanthine oxidase by allopurinol brings with it decreased formation of cytosolic reactive oxidative stress which are increased in HF, leading to decreased inhibition imposed by ROS on CK_c, which in turn stimulates the formation of cytosolic ATP from cytosolic PCr, thereby providing energy for contraction in HF. Reprinted from J Am Coll Cardiol, 59(9), Opie LH. Allopurinol for heart failure: novel mechanisms, 809-12, Copyright (2012), with permission from Elsevier.

Struthers *et al* (2002) reported that high-dose allopurinol (≥ 300 mg/day) use was associated with reduced mortality and cardiovascular events in their retrospective cohort study of 1760 chronic heart failure patients.[162] These findings were confirmed in a recent population-based cohort of 4785 heart failure patients.[163] This study suggests that we have little understanding of the determinants of exercise capacity and quality of life in chronic heart failure.

Farquharson *et al* (2002) raised the possibility that allopurinol could improve exercise capacity in patients with chronic heart failure after their study reported an improvement in endothelial dysfunction with allopurinol.[152] To answer this question, Gavin *et al.* (2005) gave three months of allopurinol 300 mg/day to 50 patients with chronic heart failure (New York Heart Association class II and II). There was significant reduction in BNP with allopurinol but no significant difference in exercise performance as assessed by modified Bruce exercise tolerance test and six-minute walk test, no significant difference in high sensitive CRP, blood pressure and health-related quality of life questionnaire.[164]

Shehab *et al* (2001) hypothesised that allopurinol-induced increases in nitric oxide bioactivity would be accompanied by favourable effects on resting heart rate variability or on spontaneous dysrhythmia counts in patients with chronic heart failure. The negative results from their small randomised, double-blind cross-over study of 16 patients with chronic heart failure did not support the hypothesis.[165]

It is not surprising that more recent studies by Hare *et al* (2008) and Givertz *et* al (2015) showed that xanthine oxidase inhibition using oxypurinol or allopurinol did not improve symptoms in patients with chronic heart failure receiving optimal medical therapy.[166,167]

The negative results from the above trials in chronic heart failure suggest that allopurinol is not helpful in the treatment of chronic heart failure. The reason for investigating allopurinol is its ability to decrease oxidative stress by reducing the production of reactive oxygen species by inhibiting xanthine oxidase. A possible explanation for the negative results is that the level of oxidative stress in patients receiving optimal medical therapy for their chronic heart failure may not be at a high enough level to show any symptomatic benefit to patients when lowering it with xanthine oxidase inhibitors.

Ischaemic heart disease

Several studies have looked at whether allopurinol could have beneficial effects in patients with ischaemic heart disease.

Rentoukas *et al* (2010) studied patients who had primary percutaneous coronary intervention (PCI) for acute ST elevation myocardial infarction (STEMI), at least 3 hours after the onset of symptoms. Allopurinol was administered as a loading dose 400 mg, followed by 100 mg for 1 month. The effects of allopurinol were more effective ST-elevation recovery, lower peak values of troponin I, creatine kinase (CK) and creatine kinase-MB (CK-MB). It was also noted that allopurinol improved clinical outcomes as a lower incidence of major cardiac events (cardiac death, non-fatal MI, repeat PCI, emergency CABG, sustained VT and intra-aortic balloon pump requirement) was present in the allopurinol group after 1 month follow-up.[168]

Similar beneficial effects were noted using the same allopurinol regimen in the study by Separham *et al* (2016) who investigated 140 patients with acute STEMI and who were candidates for thrombolytic therapy. The authors reported that allopurinol was associated with better 90-minute ST resolution, lower enzymatically determined infarct size (lower levels of CK, CK-MB and cardiac troponin I) and in-hospital major adverse cardiac events.[169]

Allopurinol offered some beneficial effects in chronic stable angina. Noman *et al* (2010) found that allopurinol had anti-anginal effects in patients with chronic stable angina. They studied patients with chronic stable angina who were given 6 weeks of allopurinol (600 mg/day). The median time to ST depression, the total exercise time and the time to angina were all increased on exercise tolerance test (Bruce protocol for treadmill test). Anti-anginal effect with allopurinol prolongs exercise in stable chronic angina. It has been suggested that the mechanism underlying allopurinol anti-anginal effects is the increases in high-energy phosphates within ischaemic tissue and the reduction of oxidative stress.[170]

Rajendra *et al* (2011) have shown that allopurinol profoundly reduces both vascular oxidative stress and endothelial dysfunction in coronary artery disease patients who are already taking optimal therapy.[154]

The protective effect of allopurinol for the cardiovascular system is further reinforced by the population-based case-control study carried by de Abajo *et al* (2015). It demonstrated that allopurinol was associated with a reduced risk of non-fatal acute myocardial infarction (mainly apparent in men when exposed to doses of $\geq$ 300mg for more than 180 days).[171]

The beneficial effects of allopurinol in ischaemic heart disease contrast with the negative results in the studies looking at chronic heart failure. A possible explanation for this is that not all chronic heart failure is due to ischaemic heart disease and in many cases, it can be due to a combination of two or more underlying cardiac diseases. It is understandably difficult to be absolutely certain that every study participant's diagnosis of chronic heart failure was due to only a particular cardiac disease.

Patients with chronic heart failure and ischaemic heart disease could also have concomittant underlying chronic lung disease which would also have an effect on the heart.

Left ventricular hypertrophy

Left ventricular hypertrophy (LVH) is associated with many diseases and is claimed to be the strongest independent predictor of cardiovascular events, cardiovascular death and total mortality.[172] Allopurinol has been shown to regress LVH in patients with chronic kidney disease[151] (CKD), ischaemic heart disease[173] (IHD) and type 2 diabetes mellitus[174] (DM) without change in blood pressure.

Kao *et al* (2011) study involved 67 patients with CKD and LVH, who were randomised to allopurinol 300 mg/day or placebo for 9 months. They found that allopurinol reduced left ventricular mass index (LVMI) by -1.42 ± 4.67 g/m^2 compared to placebo. The changes in LVMI correlated with improvement in endothelial function, suggesting that the underlying mechanism of LVH reduction is linked to afterload reduction (which is the result of improvement in endothelial function).[151]

Rekhraj *et al* (2013) had similar findings when they used higher dose of allopurinol (600 mg/day for 9 months) in patients with IHD and LVH. Allopurinol reduced LVMI by 2.2 ± 2.78 g/m^2. The authors postulated that the LVH regression was due to reduction in oxidative stress and improvement in endothelial function, hence a reduction in LV afterload.[173]

In the same year, Szejkowski *et al* (2013) published the results of their study in patients with type 2 DM and LVH. Allopurinol (600 mg/day for 9 months) reduced LVMI by 1.32 ± 2.84 g/m^2.[174]

Hypoxia and respiratory diseases

There has been an increasing interest in investigating the role of imbalance between oxidant and antioxidant factors, in particular the xanthine oxidoreductase system, in the pathogenesis of several respiratory diseases.[175] Several experimental studies have shown that allopurinol could be a possible new way to treat chronic lung diseases and pulmonary hypertension by inhibiting hypoxia-induced pulmonary vasoconstriction[176], pulmonary hypertension[176], endothelial dysfunction[177], myocardial dysfunction[178] and vascular remodeling[179].

XO activity was shown to be four times higher in sputum and bronchoalveolar lavage from patients with COPD compared with healthy subjects[180] and there was a correlation between this activity and the severity of airflow obstruction[181]. Ichinose *et al* (2003) suggested that XO contributes to the production of reactive nitrogen species (RNS) in the airway, which have a role in the inflammatory process in COPD. They gave allopurinol (300 mg/day for 4 weeks) to 10 COPD subjects and healthy subjects. They have demonstrated that reactive nitrogen species levels in the airway were elevated in COPD, and that administration of allopurinol decreased XO activity and increased exhaled nitric oxide concentration, thereby reducing airway reactive nitrogen species production.[182] Heunks *et al* (1999) reported the beneficial effects of allopurinol during strenuous exercise in patients with COPD. Exercise-induced oxidative stress was inhibited with allopurinol in this study of 16 COPD patients.[183] Unfortunately, there was no test in this study that was performed to check for evidence of pulmonary hypertension associated with COPD.

XOR is recognised as an important effector in pulmonary vascular pathology. XOR-derived free radicals cause a dose-dependent contraction of rabbit pulmonary arterial rings.[175] Terada *et al* (1992) study in cultured pulmonary artery endothelial cells showed that treatment with allopurinol inhibited the increased release of O_2^- from increased XO and XDH activity due to prolonged hypoxia.[112] Hoshikawa *et al* (2001) demonstrated elevated lung XOR activity in a model of hypoxia-induced pulmonary hypertension in which the associated pulmonary hypertensive changes (pulmonary vascular thickening) were significantly attenuated by allopurinol.[176] Jankov *et al* (2008) reported that, in the lungs of neonatal rats exposed to chronic hypoxia, the vascular remodeling induced by XO-derived reactive oxyen species was prevented by treatment with allopurinol.[179] Two more recent studies by Williams *et* al (2010) and Dopp *et al* (2011) have shown that allopurinol improved myocardial dysfunction[178] and prevented the impairment of endothelial function in skeletal muscle arteries[177] in rats exposed to chronic intermittent hypoxia.

1.7 Rationale for study

A possible new way to treat pulmonary hypertension associated with lung disease is allopurinol, which decreases both uric acid and oxidative stress. This possibility was mooted by Zharikov *et al.*[184] but not yet explored. The case for allopurinol is based on several factors. Firstly and most importantly, there are five experimental studies all showing that allopurinol inhibits hypoxia induced pulmonary vasoconstriction, pulmonary hypertension, endothelial dysfunction and vascular remodelling.[176-179,185] Secondly, hypoxia is known from many studies to up-regulate xanthine oxidase and therefore to increase its production of both uric acid and oxidative stress.[112,176] Thirdly, there is one human study where allopurinol improved endothelial function in hypoxic patients.[155] Fourthly, allopurinol profoundly reduces oxidative stress and OS is known to directly promote right ventricular hypertrophy as well as cause pulmonary vascular abnormalities.[120] There is a fifth, albeit, fairly speculative further reason for studying allopurinol in lung disease. Allopurinol blocks XO enzyme which “wastes” molecular oxygen by converting it into oxygen free radicals. Therefore, in theory blocking this oxidase should boost tissue oxygen. There are clues that allopurinol might really boost oxygen come from animal studies where allopurinol reduces myocardial oxygen consumption and a human study where allopurinol reduced ischaemia during exercise in stable angina.[158,159,170]

Therefore, there is a need to establish if allopurinol is beneficial in human lung disease in the same way as it has done in the experimental studies.

The hypothesis of this study is that allopurinol would reduce the production of reactive oxygen species, thereby decreasing oxidative stress, which would lead to regression of right ventricular hypertrophy in patients with pulmonary hypertension associated with chronic lung disease.

2. Methods

2.1 Study Design

2.1.1 Study Overview

This is a single centre randomised, double-blind, placebo-controlled, parallel-group study which was funded by the British Heart Foundation (reference: PG/14/6/30592). The study was approved by East of Scotland Research Ethics Committee on the 11th August 2014 (reference: 14/ES/1035), and by the Medicines and Healthcare products Regulatory Agency (MHRA) on 21st January 2015 (Eudract number: 2014-002305-38). The trial is registered on the International Standard Registered Clinical/social sTudy Number (ISRCTN) registry (reference: ISRCTN11081180). This study was carried out in accordance with the Declaration of Helsinki. The study was conducted between April 2015 and July 2017 at Ninewells hospital, Dundee, Scotland. All participants provided written informed consent after receiving a patient information sheet at least twenty-four hours before.

2.1.2 Study Objectives

The main aim of this study is to establish if allopurinol can regress right ventricular hypertrophy in patients with pulmonary hypertension associated with chronic obstructive pulmonary disease or interstitial lung disease. The study acronym is the ALPHA trial (does Allopurinol reduce right ventricuLar mass in Pulmonary Hypertension Associated with chronic lung disease). The secondary objectives were to assess the effect of allopurinol on cardiac size and function measured by magnetic resonance imaging (right ventricular end diastolic volume, right ventricular end systolic volume, right ventricular ejection fraction, left ventricular mass, left ventricular end diastolic volume, left ventricular end systolic volume, left ventricular ejection fraction, pulmonary artery wave velocity), on exercise capacity as measured by six minute walk test (6MWT), on oxygen saturation pre and post 6MWT, on quality of life measures (St. George's Respiratory Questionnaire, 36-Item Short Form survey, Transition Dyspnoea Index and King's Brief Interstitial Lung Disease questionnaire), and on blood markers (N-terminal prohormone of brain natriuretic peptide and high-sensitivity troponin I).

2.2 Study population

72 participants were recruited between April 2015 and July 2016. The inclusion and exclusion criteria used in the study are illustrated in *Figure 19*.

Inclusion criteria

- Male or female participants aged 18 years or over
- Known diagnosis of chronic obstructive pulmonary disease or interstitial lung disease
- Stable lung disease medication for at least two weeks prior to consent
- Pulmonary acceleration time < 110 ms and/or right ventricular systolic pressure > 25 mm Hg and/or right ventricular free wall thickness ≥ 5.5 mm (on screening echocardiography)

Exclusion criteria

- Documented allergy or intolerance to allopurinol
- Active gout (i.e flare up < 2 years) or currently taking allopurinol
- Left ventricular ejection fraction < 45% on echocardiography
- Severe aortic stenosis on echocardiography
- Severe hepatic disease
- Chronic kidney disease class 3B or greater
- Patients taking azathioprine, 6-mercaptopurine, or theophylline
- Malignancy (receiving active treatment) or other life-threatening diseases
- Any contraindication to MRI (claustrophobia, metal implants)
- Unable to give informed consent

Figure 19 - Inclusion & exclusion criteria for study

2.2.1 Recruitment

The participants were recruited from five separate sources identified as:

1) NHS Tayside chest clinics
2) The local clinical COPD database in Tayside
3) The local respiratory research databases
4) The general practices covering two UK National Health Service boards (Tayside and Fife) via the Scottish Primary Care Research Network (SPCRN)
5) Scottish Health Research Register (SHARE)

2.2.2 Scottish Primary Care Research Network

SPCRN is the national primary care research network in Scotland and was established in 2002.[186] It is funded by the Chief Scientist Office to facilitate high quality, funded, research studies. SPCRN was used to boost recruitment for the study within GP practices in Dundee, Angus and Fife. In the first instance, the SPCRN staff have sent an invitation email to general practitioners to ask if they would be interested to help with recruitment of the study. They then went to the GP surgeries who have agreed and they identified potentially eligible patients via the GP surgery computer database searches using the study eligible criteria. The list was then reviewed by one of the GP partners to exclude any unsuitable patients. SPCRN then helped to prepare the ethically approved letters to be sent out to eligible patients.

2.2.3 Scottish Health Research Register

SHARE is an initiative of NHS Research Scotland. It is a partnership between the National Health Service, the Scottish government and universities in Scotland to establish a Scottish register of people interested in participating in medical research.[187] Patients who have signed up to the register, agree to allow SHARE to use the coded data in their various NHS computer records to check whether they might be suitable for health research studies. After doing NHS electronic database searches using coded data, staff from SHARE have contacted potentially eligible patients to ask them if they would be interested to participate in the study. If they were interested, their contact details would then be passed to PLSC to arrange an appointment.

2.3 Study visits

After they were recruited, the participants were enrolled in the study for a period of between 9 to 13 months. They attended for nine study visits including two telephone calls. A detailed study schedule is outlined in *Figure 20*.

A screening visit was carried out for all participants to assess whether they fulfilled the entry criteria for the study. The participants were all consented prior to screening. At the screening visit, after informed consent, a medical history was taken and a clinical examination was performed. The participants then had an echocardiogram to check for evidence of pulmonary hypertension. Once the patients were known to be eligible, they had blood samples taken for safety analysis, and their vital signs, height and weight were measured. They also had a practice six minute walk test on the same day.

They returned for the randomisation visit at any time up to four weeks after the initial screening visit. At the randomisation visit, they completed a six minute walk test, quality of life questionnaires and had their vital signs and pulmonary function tests measured. They also had their cardiac magnetic resonance imaging (CMRI) scan done on that day or within two weeks of this study visit. They were then randomly assigned to either placebo or allopurinol 100 mg.

The participants were reviewed at two weeks, six weeks, three-month and nine-month. Safety blood tests were done, vital signs were measured, and compliance was checked and documented at each of the visits. The participants were advised on the importance of correct compliance if the compliance was poor, aiming for a compliance of above seventy percent.

Two telephone call visits were done at 8 weeks and 6-month. Any changes to their concomitant medications and any adverse events were followed up. The participants were reminded the importance of compliance.

The participants continued all their usual medication, which remained unchanged throughout the study unless clinically indicated. When the participants were suffering from acute exacerbations of their respiratory condition (COPD or ILD), they were treated as per usual practice and the allopurinol or placebo was not stopped.

A maximum of three out of nine visits (screening, randomisation and final visits) were done at Ninewells Hospital. The remaining study visits were offered to be done at the participant’s home at their convenience.

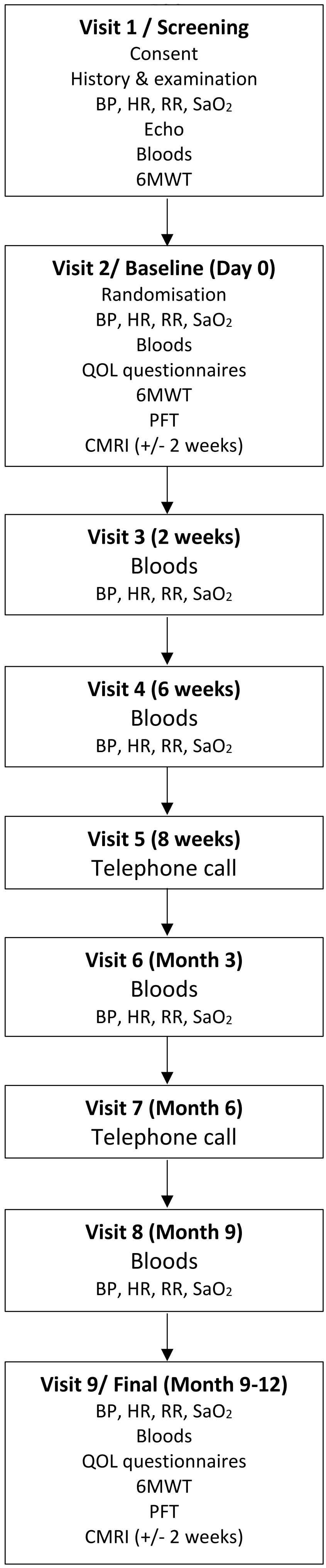

Figure 20 - Study visits

2.3.1 Safety blood tests

Screening blood samples were taken for routine blood tests including full blood count (FBC), renal function (U&E), liver function (LFT), uric acid (UA), random blood glucose, haemoglobin A1C (if patient has a diagnosis of diabetes mellitus), lipid profile. These blood tests were repeated at final visit. Routine safety blood tests (FBC, U&E, LFT) were taken at regular visits throughout the study. The participants were reviewed at two weeks, six weeks, three month and nine month for these safety blood tests. The safety blood samples were sent to and analysed by the Blood Sciences laboratory at Ninewells Hospital.

2.3.2 Research blood tests

Blood samples were taken at the screening visit and the final visit for research blood tests. Venous blood was drawn into ethylenediaminetetraacetic acid (EDTA) and serum blood bottles. The EDTA bottles were immediately kept on ice and centrifuged as soon as possible for ten minutes at 3000 rpm at 4^0C (Heraeus Megafuge 1.0R, Kendro, Germany). The plasma was then pipetted into 5ml plastic aliquot bottles. The blood in the serum bottles was allowed to clot for fifteen minutes at room temperature before centrifuging for ten minutes at 3000 rpm at 4^0C. The serum was then pipetted into 5ml plastic aliquot bottles. Aliquot bottles for both serum and plasma were stored at -80^0C. An additional blood sample was taken from the participants and stored in a secure laboratory at -20^0C for future genetic research if the participants have previously agreed.

N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was measured from plasma using a multi-array assay system (Meso Scale Discovery, USA). High-sensitivity troponin I (hs-Trop I) was measured from serum using a digital immunoassay on the SIMOA HD1-Analyser (Quanterix, USA).

2.3.3 Echocardiography

At the screening visit, the participant had a transthoracic echocardiography (echo) to assess if they fulfilled the criteria for pulmonary hypertension and to exclude impaired left ventricular systolic function and significant valve disease. The study was carried out using a Philips iE33 system (Philips, Netherlands) or a Philips Epiq7 ultrasound system (Philips Ultrasound, USA). If measurements were difficult to obtain using standard acoustic windows, we used the oblique subcostal windows[188] to assess the pulmonary acceleration time with pulse-wave Doppler. The study protocol stated that patients were excluded if they had an estimated left ventricular ejection fraction of < 45%. For practical reasons, the technique of visual estimation was used to estimate LVEF rather than a quantitative measurement. We calculated the estimated mean pulmonary arterial pressure with the Dabestani equation[189], and the right ventricular systolic pressure with the Bernoulli equation.

2.3.4 Vital signs

Vital signs were measured at each study visit.

- Blood pressure (BP) was performed using an automated sphygmomanometer (Omron 705IT, Omron Healthcare Europe, The

Netherlands). All measurements were performed in a seated position with at least 10 minutes rest before the BP was recorded.

- Resting peripheral oxygen saturation (SaO_2) and heart rate (HR) were measured using a finger pulse oximeter (Merlin Medical Ltd, UK).
- Respiratory rate (RR) was measured by counting the number of breaths over a minute.

2.3.5 Spirometry

Spirometry was performed by PLSC and LC using a Micro Medical Microloop spirometer (Viasys Healthcare, Micro Medical Limited, Kent) according to the ATS/ERS Task Force standardisation of spirometry document[190]. All patients were confirmed to have taken their usual inhaled therapy prior to performing spirometry. Spirometry was done at the baseline visit and the final visit.

2.3.6 Six Minute Walk Test

Six minute walk tests were performed at the screening visit, the baseline visit and the final visit. On each of these occasions, the 6MWT was carried out on an even floor with vocal support from the investigator/delegate which is in full accord with ATS guidelines.[191] The one done at the screening visit was a practice 6MWT and the one done at baseline visit was used for the baseline analysis. The 6MWT is fairly reproducible in lung disease with a correlation coefficient of 0·88.[192] As is traditional and recommended for lung studies of the 6MWT, the distance achieved was taken as the main result. Oxygen saturation (SaO_2) was also measured during the 6MWT with a portable pulse oximeter (Merlin Medical Ltd, UK). Patients who were on long term oxygen therapy (LTOT) were included as there was no reason to believe that LTOT would alter whether allopurinol works or not. It would be good to make this study relevant to all lung disease/PH patients so that any guideline advice that comes from this study will not only then apply to a subset of patients. The participants on LTOT had done their six minute walk test off oxygen (O_2). This is because patients on LTOT spend nine hours every day off oxygen anyway and this is particularly likely when they are mobile. Therefore, by having them off O_2 during the 6MWT mimics how they would be when they try to become mobile.

2.3.7 Quality of Life measures

Quality of life (QOL) measures were carried out at baseline and final visits. The disease specific QOL measure which is used in COPD studies is the St George's Respiratory Questionnaire (SGRQ). This is because it has been validated specifically in COPD patients: its subscales have reliabilities in the 0·78-0·85 range [193] and it is responsive to change.[194] The minimal important difference (MID) is a change of 4 units. The baseline (BDI) and transition (TDI) dyspnoea indices are commonly used methods to assess breathlessness and the impact of intervention.[195] BDI was recorded at baseline visit and TDI at final visit. The TDI has a MID of 1 unit.

It is traditional in QOL assessment to use both a general QOL measure and disease specific questionnaire in a study. For that reason this study also used the 36-Item Short Form Survey (SF-36). This is again for several reasons: it is the general QOL measure which is most used in COPD studies, it is reliable, it reflects disease severity and it is responsive.[193,196-198] Only participants with ILD completed the King's Brief Interstitial Lung Disease (K-BILD) QOL questionnaire which is a measure of health status for people with ILD.[199,200]

2.3.8 Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging (CMRI) was performed at the baseline and at the final visit only. MRI safety was established prior to the scan. This included: assessing for in vivo ferrous material, claustrophobia, abnormal renal function and pregnancy as per local NHS Radiology Safety Procedures. CMRI images were acquired on a 32 RF cardiac receiver channel, 3 Tesla MRI scanner (Prisma, Siemens, Erlangen, Germany) using dedicated phase array cardiac and phosphorous spectroscopy coils Serial contiguous short-axis cines were acquired from the vertical long axis and horizontal long axis of the left ventricle (electrocardiogram gated, steady-state free precession imaging [true fast imaging with steadystate precession], with the short axis imaging parameters being a repetition time of 2.5ms, echo time of 1.1ms, flip angle of 60°, and slice thickness 6mm). The images were exported and the analysis was performed offline by a single independent, blinded radiologist (JWM) using CVI 42 (Circle Cardiovascular Imaging software, Calgary, Canada) for assessment of ventricular volumes (EDV, ESV, stroke volume), EF, and ventricular mass. Ventricular mass and volumes were also index to body surface area, which were calculated using the Mosteller formula.[201] The reproducibility of the right ventricular mass assessment using MRI was derived for this observer.

2.4 Randomisation

After successful screening for eligibility and safety, the participants were randomised to either allopurinol or placebo in a double blind fashion. Double blind medication (allopurinol or placebo) were prepared, packaged and labelled by Tayside Pharmaceuticals.

Randomisation were carried out by Tayside Pharmaceuticals using block randomisation in twelve groups of six (with three active/three placebo in each block). They used a validated randomisation program and had securely backed up both the randomisation seed and the randomisation allocation. A copy of the allocation was supplied with the allopurinol and placebo tablets to the Clinical Trials Pharmacy at Ninewells Hospital who operates a 24 hour emergency unblinding facility.

2.4.1 Drug titration

The participants were dosed with either allopurinol 100 mg or matched placebo at the randomisation visit. They were asked to take one tablet daily for two weeks. Thereafter, the medication was increased to 300 mg once daily or placebo and continued for four weeks. If the study drugs were tolerated, the dose was increased after this four week period to the target dose of 600 mg allopurinol/placebo (given as one 300 mg tablet twice daily) and continued for a further 7.5 to 10.5 months so that the participant was in the trial, on medication, for 9 to 12 months.

If there was any significant deterioration in the safety blood tests, the dose was then stopped and the participant did not receive any further IMP. If the participants had tolerated a lower dose (300 mg) and was experiencing side effects on the higher dose (600 mg), they were downtitrated back to the 300mg dose. If the participant was taken off the study, their GP and consultant was informed of the abnormal blood results or the reason for stopping the trial medication. If the trial medication was stopped, the participant remained in the study for the entire duration if they had agreed to it.

2.4.2 Study drug

The manufacture, packaging and labelling of the study drug was carried out by Tayside Pharmaceuticals, Ninewells Hospital, Dundee. Allopurinol was enclosed in a hard gelatine capsule shell. The placebo capsules had an identical appearance to the active drug and contained microcrystalline cellulose. Allopurinol/placebo capsules were included in packs containing the adequate number of units required for each treatment period. The study medication was stored under the supervision of the Clinical Trials Pharmacist at the Clinical Trials Pharmacy at Ninewells Hospital. The temperature logs were maintained throughout the study and were reviewed as part of the monitoring plan.

2.4.3 Prohibited medications

The concurrent prescription of 6-mercaptopurine, azathioprine or theophylline was not allowed due to the known interaction of these drugs with allopurinol. Any participants already on these drugs were excluded at the screening visit.

The use of ampicillin or amoxicillin was not prohibited. An increase in frequency of skin rash has been reported among patients receiving ampicillin or amoxicillin concurrently with allopurinol compared to patients who are not receiving both drugs. The cause of the reported association has not been established. However, in participants receiving allopurinol an alternative to ampicillin or amoxicillin was used where available.

2.4.4 Emergency unblinding procedures

A clinician familiar with the research was available each workday for contact by all participants. Any other clinician who saw the participants in the study was free to stop the study drug if they felt it was clinically indicated and they would have contacted the Clinical Trials Pharmacy Department at Ninewells Hospital to break the code if they felt it was necessary. Emergency unblinding was not needed throughout the duration of the study.

2.4.5 Withdrawal procedures

The participants were given the choice as to whether they withdrew or not from the study if they suffered side effects from the medication. Rash is known to be the main side effect and withdrawal would occur if it was marked as persistent.

The participants who withdrew would be replaced if possible within the study timeframe. Those who withdrew from study drugs were encouraged to attend future study visits and where possible the final visit for outcome measures. PLSC maintained contact with those who withdrew to ensure resolution of adverse event(s). The reasons for withdrawal were noted in the participant's case report form (CRF) and case notes.

The participants were free to withdraw from the study at any time. The reasons, if known, were recorded in the medical case notes and CRF.

2.5 Pharmacovigilance

The study was conducted in accordance with the principles of Good Clinical Practice (GCP). The trial was monitored in accordance with TASC SOP 04 to ensure adherence to GCP.

Patient contact was frequent during the trial (7 face-to-face visits and 2 telephone calls) to ensure that there was no safety concern. At each patient contact, all reported adverse events were recorded in detail (dates, expected causation and action taken) on an adverse event (AE) CRF page. The seriousness of the AE was assessed by the principal investigator (PLSC) who would initiate the appropriate treatment if required. Events such as acute chest infections and increased dyspnoea were not recorded or reported as they are common events in this patient population.

All AEs which were classified as serious adverse events (SAEs) were reported to the pharmacovigilance section of TASC (pharmacovigilance.tayside@nhs.net). PLSC completed a SAE report form for each one of them. He has assessed for expectedness and causality, based on the knowledge of the reaction and the relevant product information.

All AEs and SAEs were recorded from the time a participant consents to join the study until their last study visit. The participants with unresolved AEs at the last study visit were followed up until resolution or 30 days after last study visit, whichever is sooner.

2.6 Data collection & management

The data was initially collected by PLSC and LC on a paper case report form (CRF). The CRFs were stored in a locked filling cabinets in a room with door locked out-of-hours. The access to the CRFs was only available to PLSC and LC. The data was subsequently transcribed to an electronic CRF on a web-based freeware database (OpenClinica, USA).

The data management system was provided by Tayside Clinical Trials Unit (TCTU) using OpenClinica (OC). The system was based on the study protocol and the CRF. The development and validation of the study database, the quality control and the extraction of the data were done according to TCTU procedures.

The MRI data was stored on NHS Tayside Clinical Systems and was anonymised by a single blinded observer (JWM) before being analysed at end of the trial.

2.7 Statistics and data analysis

2.7.1 Sample size calculation

66 patients were required to achieve 80% power to detect a 5 g change in RV mass at a significance level of $p < 0.05$, based on previous studies [151,173,174] investigating the allopurinol's effect on (left) ventricular mass using CMRI. In order to allow for 10% drop-outs, 72 eligible patients were recruited.

One review article suggests that the initial proof of concept study (like this) should only involve 30-40 patients.[202] Another authoritative article from a leading expert in England suggests that 34 patients are required to have 80% power at $p < 0{\cdot}05$ to detect changes in the manually measured outcomes of RV mass of 10 g.[5] However, a 10 g change in ventricular mass was over ambitious. Sildenafil, which is an established treatment for PH, only reduces RV mass by 8 g in pulmonary arterial hypertension.[203]

2.7.2 Statistical analysis

The data for continuous outcome measures have be assessed for normality prior to analysis. The descriptive statistics in form of mean ± standard deviation (SD) for normally distributed continuous variables, median and interquartile range (IQR) for non-normally distributed continuous variables, and percentages and denominators for categorical variables are tabulated at the baseline visit. The comparison between continuous variables are analysed using paired t-tests (to test within group differences), independent t-test (to test between group differences), or Mann-Whitney U test (for non-normally distributed data) whilst categorical variables were analysed using Chi-squared test.

All statistical analyses were undertaken blinded using IBM SPSS Statistics v22.0 (IBM, United States). A two-sided p value < 0.05 was considered statistically significant.

2.7.3 Missing data

The primary analysis was based on the intention-to-treat principle. The extent of missing data was examined and the reason for drop-out was ascertained. Multiple imputation was used to impute missing values and where assumptions for missing at random data were met. Complete case analysis where missing patients are excluded was carried out as a secondary analysis.

3. Results

3.1 Study Recruitment

A total of 1914 invitation letters were sent from April 2015 to June 2016. 422 (22%) replied that they were interested. After reviewing electronic medical records, 231 were excluded from invitation if eligibility criteria were not met or if patient declined after phoning them. A total of 191 participants were screened from April 2015 to July 2016. Recruitment was halted for six weeks from end of September to October 2015 due to the MRI machine being serviced and upgraded. 119 participants, out of 191 screened, were excluded because of the following reasons:

- 91 participants did not meet the echo criteria for pulmonary hypertension
- 17 participants were unable to undergo CMRI
    - Contraindications to MRI (n = 7)
    - Claustrophobia (n = 7)
    - Unable to tolerate MRI (n = 3)
- 11 participants had other reasons:
    - Recent change of medication within two weeks of screening (n = 2)
    - Already enrolled in another clinical trial (n = 2)
    - On theophylline (n = 1)
    - New diagnosis of lung cancer (n = 1)
    - Deranged liver function tests (n = 1)

- Aortic valve disease (n = 1)
- Chronic kidney disease stage 3B (n = 1)
- Participant's decision to withdraw (n = 1)
- Surplus to requirement (n = 1)

72 participants were randomised. 36 participants were recruited to the allopurinol arm and 36 participants to the placebo arm of the trial. Eight participants withdrew from the study (five from the allopurinol group and three from the placebo group). The reasons for withdrawing from the trial included: participant's preference (n = 4), depression (n = 2), metastatic bladder cancer (n = 1) and death (n =1).(*Figure 18*) None of the withdrawals were felt to be related to the study medication. One participant who was randomised to the placebo group was excluded from analysis because he was unable to undergo baseline CMRI because of claustrophobia. Another participant who was randomised to the allopurinol group, had undergone baseline CMRI but declined to undergo repeat CMRI at final visit, was included in the analysis.

3.2 CONSORT diagram

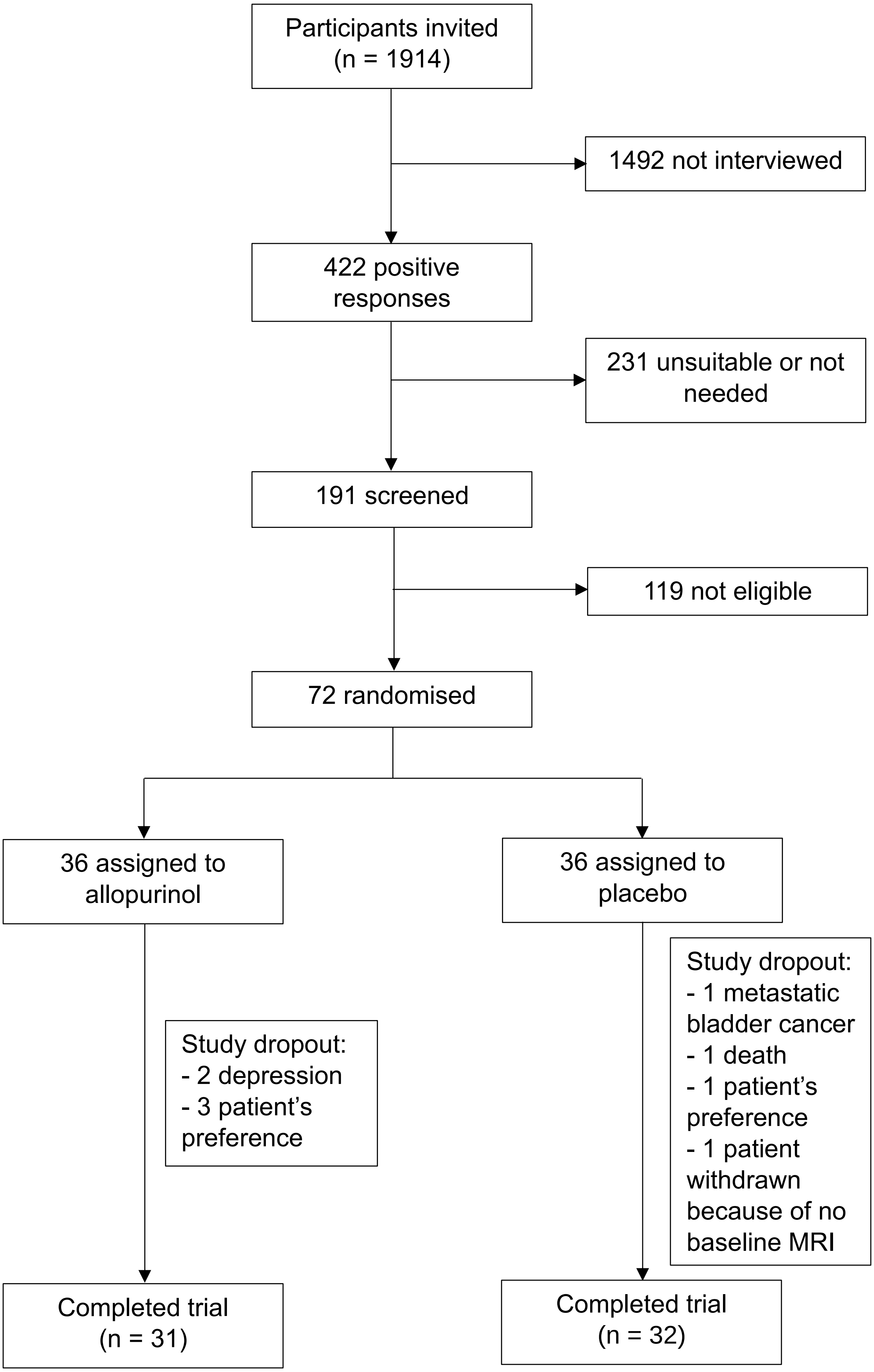

Figure 21 - CONSORT diagram

3.3 Baseline Characteristics

The baseline characteristics of the participants are outlined in *Table 3*. The study participants had a mean age of 71 years, estimated MPAP 30 mm Hg, mean FEV_1 60% predicted, resting SaO_2 96% and were predominantly male (62%). There were no significant differences between the groups for the baseline characteristics. There was no significant difference in baseline uric acid level, RVM/RVMI measured using CMRI, NT-ProBNP level and hs-Trop I level. 66 (93%) participants had COPD and 5 (7%) had IPF. 5 patients were on long-term oxygen therapy (LTOT).

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Age (years)	70 (5)	71 (6)	0.45
Male sex (%)	22 (61)	22 (63)	
Height (m)	1.65 (0.11)	1.68 (0.09)	0.29
Weight (kg)	80.2 (16.6)	82.7 (17.0)	0.54
BMI (kg/m^2)	29 (5)	29 (5)	0.98
Heart rate (bpm)	79 (14)	78 (13)	0.99
Systolic BP (mm Hg)	138 (16)	140 (19)	0.67
Diastolic BP (mm Hg)	76 (9)	75 (14)	0.93
mMRC dyspnoea scale	2.8 (1.3)	2.7 (1.2)	0.14
WHO Functional class	2.3 (0.8)	2.4 (0.7)	0.48
Smoking status • Current smoker • Ex-/non-smoker	 9 (25) 27 (75)	 8 (23) 27 (77)	
Pack-year history	46.9 (27.4)	50.3 (28.6)	0.61
Long-term oxygen	3 (8)	2 (6)	
SaO2 (%)	96 (3)	96 (3)	0.67
PAT (ms)	94.9 (9.6)	97.1 (12.1)	0.38

Table 5 - Baseline characteristics

Data are mean (SD), n (%) or median (IQR), unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: BMI = body mass index; mMRC = modified medical research council; SaO_2 = blood oxygen saturation

3.3.1 Past medical history and concomittant medications

Past medical history / concomitant medications	**Allopurinol (n = 36)**	**Placebo (n = 35)**
COPD	34 (94%)	32 (92%)
ILD	2 (6%)	3 (8%)
Ischaemic heart disease	17 (47%)	5 (14%)
Hypertension	20 (56%)	20 (57%)
Hypercholesterolaemia	22 (61%)	21 (60%)
Cerebrovascular disease	7 (19%)	4 (11%)
Type 2 diabetes mellitus	8 (22%)	6 (17%)
Peripheral vascular disease	5 (14%)	3 (9%)
LABA	29 (81%)	27 (77%)
LAMA	26 (72%)	27 (77%)
ICS	19 (53%)	18 (56%)
Beta-blocker	6 (17%)	6 (17%)
ACE inhibitor	10 (28%)	11 (31%)
ARB	4 (11%)	3 (9%)
Statin	23 (64%)	21 (60%)
Diuretic	9 (25%)	9 (26%)

Table 6 - Past medical history and concomitant medications

Data are n (%) unless otherwise indicated.

Abbreviations: COPD = chronic obstructive pulmonary disease; ILD = interstitial lung disease; LABA = long-acting beta-agonist; LAMA = long-acting muscarinic antagonist; ICS = inhaled corticosteroid; ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blocker

3.3.2 Baseline spirometry

COPD	**Allopurinol (n = 34)**	**Placebo (n = 32)**	**p**
FEV_1 (L)	1.45 (0.65)	1.46 (0.66)	0.95
FEV_1 (% predicted)	60 (21)	58 (20)	0.64
FVC (L)	2.78 (0.99)	2.94 (0.94)	0.52
FVC (% predicted)	90 (18)	92 (21)	0.62
FEV_1/FVC (%)	52 (14)	49 (13)	0.41
FEF_{25-75}	0.62 (0.33)	0.60 (0.37)	0.83
FEF_{25-75} (% predicted)	22 (11)	21 (11)	0.64
GOLD 1	9 (26)	3 (9)	
GOLD 2	16 (47)	16 (50)	
GOLD 3	6 (18)	12 (38)	
GOLD 4	3 (9)	1 (3)	

Table 7- Baseline spirometry and GOLD stage for patients with COPD

Data are mean (SD), median (IQR) or n (%), unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: FEV_1 = forced expiratory volume in 1 sec; FVC = forced vital capacity; FEF_{25-75} = forced expiratory flow 25-75%

ILD	Allopurinol (n = 2)	Placebo (n = 3)	p
FEV_1 (L)	2.51 (0.27)	1.94 (0.71)	0.37
FEV_1 (% predicted)	94 (6)	70 (24)	0.29
FVC (L)	3.15 (0.42)	2.71 (0.81)	0.55
FVC (% predicted)	91 (6.4)	81 (32)	0.71
FEV_1/FVC (%)	80 (2)	71 (9)	0.30
FEF_{25-75}	2.12 (0.36)	1.35 (0.83)	0.32
FEF_{25-75} (% predicted)	71 (11)	44 (29)	0.33

Table 8 - Baseline spirometry for patients with ILD

Data are mean (SD) or median (IQR), unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: FEV_1 = forced expiratory volume in 1 sec; FVC = forced vital capacity; FEF_{25-75} = forced expiratory flow 25-75%

3.3.3 Baseline blood results

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Haemoglobin (g/dL)	141	142	0.81
Haematocrit (l/L)	0.43	0.43	0.93
Glucose (mmol/L)	6.3 (2.1)	5.7 (1.8)	0.20
HDL-C (mmol/L)	1.3 (0.5)	1.5 (0.5)	0.16
GFR (ml/min)	96.1 (24.9)	91.8 (27.7)	0.49
Eosinophil (x 10^9/L)	0.22 (0.15)	0.26 (0.14)	0.25
Neutrophil (x 10^9/L)	4.95 (1.36)	5.59 (1.46)	0.06
Lymphocyte (x 10^9/L)	1.90 (0.68)	2.16 (0.64)	0.10
Uric acid (mmol/L)	350.1 (81.3)	342.9 (90.9)	0.73
NT-proBNP (pg/ml)	918 (1491)	949 (1627)	0.93
Hs-Trop I (pg/ml)	3.2 (3.3)	3.6 (4.3)	0.69

Table 9 - Baseline blood results

Data are mean (SD), n (%) or median (IQR), unless otherwise indicated. Peason’s chi-square was used for discrete variables, independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: HDL-C = high density lipoprotein cholesterol; GFR = glomerular filtration rate; NT-proBNP = N-terminal prohormone brain natriuretic peptide; Hs-Trop I = high sensitive troponin I.

3.3.4 Baseline CMRI measurements

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
RVM (g) Normal: 28 - 49	40.27 ± 1.62	41.19 ± 2.06	0.73
RVMI (g/m^2) Normal: 16 - 25	21.02 ± 0.68	20.88 ± 0.83	0.90
RVESV (ml) Normal: 39 – 98	49.7 ± 2.9	55.8 ± 3.6	0.19
RVEDV (ml) Normal: 108 - 217	118.6 ± 5.2	129.1 ± 5.8	0.17
RVSV (ml) Normal: 66 - 125	68.8 ± 3.3	73.4 ± 3.8	0.35
RVEF (%) Normal: 56 - 67	57.0 ± 2.0	56.5 ± 1.7	0.85
LVM (g) Normal: 77 – 155	106.8 ± 4.8	105.9 ± 4.9	0.89
LVMI (g/m^2) Normal: 51 - 76	55.5 ± 1.8	53.7 ± 2.0	0.52
LVSV (ml) Normal: 72 – 126	70.9 ± 3.4	76.3 ± 3.3	0.25
LVEF (%) Normal: 62 - 72	52.9 ± 1.9	56.8 ± 1.5	0.10

Table 10 - Baseline cardiac MRI measurements

Data are mean ± SD, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index; RVESV = right ventricular end-systolic volume; RVEDV = right ventricular end-diastolic volume; RVSV = right ventricular stroke volume; RVEF = right ventricular ejection fraction; LVM = left ventricular mass, LVMI =

left ventricular mass index; LVSV = left ventricular stroke volume; LVEF = left ventricular ejection fraction.

Normal values were taken from Kawel-Boehm N *et al.* review.[204]

3.4 Effect of Allopurinol on RVM and RVMI

71 patients underwent CMRI (allopurinol, n = 36; placebo, n = 35). At baseline, the right ventricular mass (RVM) and the right ventricular mass index (RVMI) were similar between the two groups. After 12 months of allopurinol therapy, in the intention-to-treat analysis, there was no significant difference in the change in RVM (allopurinol group 1.85 ± 1.56 g vs. placebo group 0.97 ± 1.20 g; p = 0.66) and in the change in RVMI (allopurinol group 0.70 ± 0.75 g/m^2 vs. placebo group 0.50 ± 0.60 g/m^2; p = 0.83) between the two groups (*Table 11*). Analysis was also done on completed cases and there was no significant difference in change in RVM or in RVMI. Bonferroni correction was used to make adjustment for multiple comparisons.

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline RVM (g)	40.27 ± 1.62	41.19 ± 2.06	0.73
Final RVM (g)	42.13 ± 1.42	42.16 ± 1.99	0.99
Change in RVM (g)	1.85 ± 1.56	0.97 ± 1.20	0.66
Baseline RVMI (g/m^2)	21.02 ± 0.68	20.88 ± 0.83	0.90
Final RVMI (g/m^2)	21.73 ± 0.57	21.39 ± 0.82	0.73
Change in RVMI (g/m^2)	0.70 ± 0.75	0.50 ± 0.60	0.83

Table 11 - Effect of allopurinol on RVM and RVMI (intention-to-treat analysis)

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index

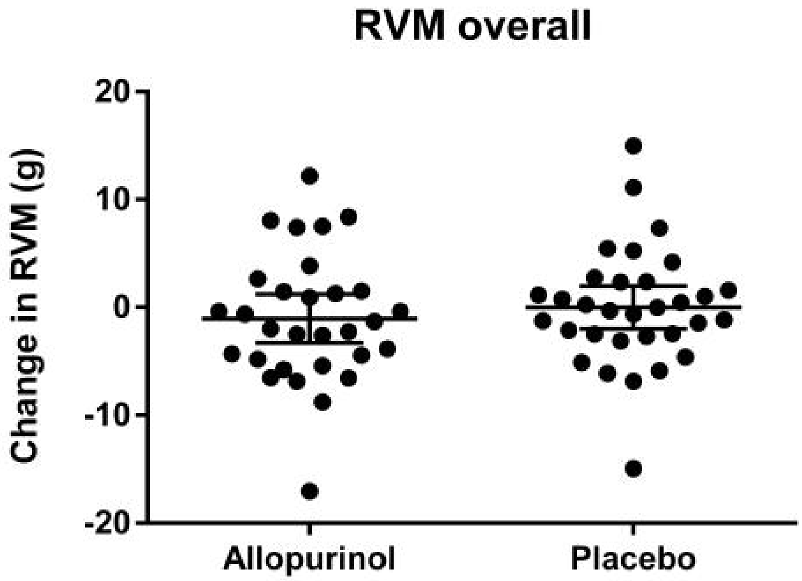

Figure 22 - Change in RVM

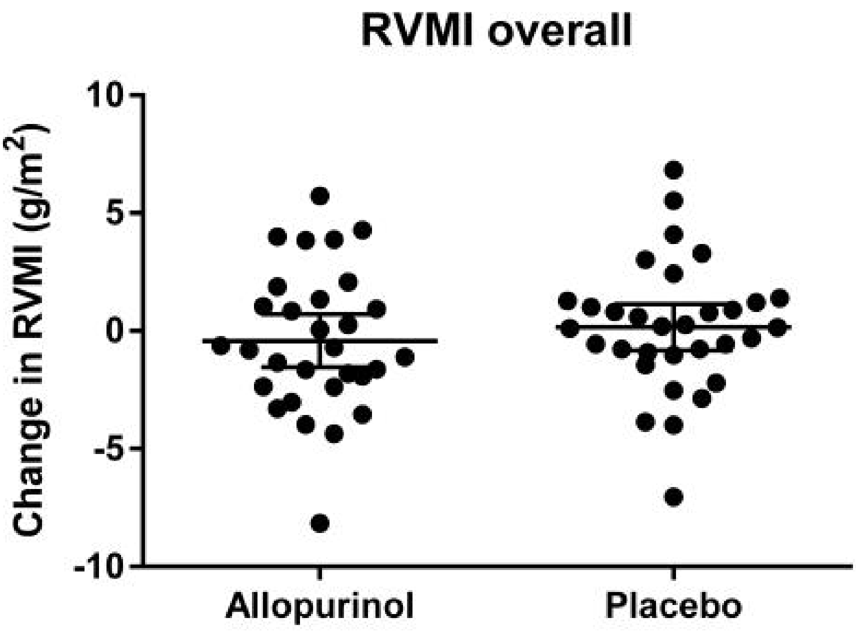

Figure 23 - Change in RVMI

	Allopurinol (n = 30)	Placebo (n = 32)	p
Baseline RVM (g)	41.4 ± 10.2	39.7 ± 13.2	0.47
Final RVM (g)	39.1 ± 13.2	41.4 ± 11.9	0.99
Change in RVM (g)	-1.7 ± 6.8	0.0 ± 4.9	0.30
Baseline RVMI (g/m^2)	22.2 ± 4.7	20.6 ± 6.4	0.18
Final RVMI (g/m^2)	21.6 ± 3.8	20.8 ± 5.4	0.39
Change in RVMI (g/m^2)	-0.8 ± 3.8	0.2 ± 2.2	0.25

Table 12 - Effect of allopurinol on RVM and RVMI (complete-cases analysis)

Data are presented as Median ± Interquartile range. Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index

3.5 Effect of Allopurinol on parameters measured on CMRI

For the other parameters measured on cardiac MRI for both right and left ventricles, there were no significant changes in end-systolic volume (ESV), end-diastolic volume (EDV), ejection fraction (EF), stroke volume (SV) and their index values.

3.5.1 Effect of Allopurinol on Right Ventricular volumes

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline RVESV (ml)	49.7 ± 2.9	55.8 ± 3.6	0.19
Final RVESV (ml)	54.5 ± 2.7	59.5 ± 4.9	0.37
Change in RVESV (ml)	4.8 ± 2.5	3.8 ± 2.8	0.79
Baseline RVESVI (ml/m^2)	26.1 ± 1.6	28.3 ± 1.7	0.33
Final RVESVI (ml/m^2)	28.0 ± 1.2	30.1 ± 2.3	0.43
Change in RVESVI (ml/m^2)	1.9 ± 1.3	1.7 ± 1.4	0.92
Baseline RVEDV (ml)	118.6 ± 5.2	129.1 ± 5.8	0.17
Final RVEDV (ml)	127.4 ± 3.6	134.7 ± 6.3	0.31
Change in RVEDV (ml)	8.8 ± 4.2	5.6 ± 4.2	0.59
Baseline RVEDVI (ml/m^2)	62.2 ± 2.5	65.5 ± 2.3	0.34
Final RVEDVI (ml/m^2)	66.3 ± 1.7	68.6 ± 2.5	0.45
Change in RVEDVI (ml/m^2)	4.0 ± 2.2	3.1 ± 2.2	0.75

Table 13 - Effect of allopurinol on RVESV, RVESVI, RVEDV and RVEDVI

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVESV = right ventricular end-systolic volume; RVESVI = right ventricular end-systolic volume index; RVEDV = right ventricular end-diastolic volume; RVEDV = right ventricular end-diastolic volume index.

3.5.2 Effect of Allopurinol on Right Ventricular Stroke Volume & Ejection Fraction

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline RVSV (ml)	68.8 ± 3.3	73.4 ± 3.8	0.35
Final RVSV (ml)	71.8 ± 2.5	75.0 ± 3.5	0.46
Change in RVSV (ml)	3.0 ± 3.1	1.6 ± 2.7	0.73
Baseline RVSVI (ml/m^2)	36.1 ± 1.7	37.2 ± 1.5	0.62
Final RVSVI (ml/m^2)	37.5 ± 1.3	38.3 ± 1.5	0.69
Change in RVSVI (ml/m^2)	1.4 ± 1.7	1.1 ± 1.3	0.88
Baseline RVEF (%)	57.0 ± 2.0	56.5 ± 1.7	0.85
Final RVEF (%)	58.3 ± 1.8	58.2 ± 2.1	0.95
Change in RVEF (%)	1.3 ± 2.4	1.7 ± 1.7	0.91

Table 14 - Effect of allopurinol on RVSV, RVSVI and RVEF

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVSV = right ventricular stroke volume; RVSVI = right ventricular stroke volume index; RVEF = right ventricular ejection fraction.

3.5.3 Effect of Allopurinol on Left Ventricular Mass

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline LVM (g)	106.8 ± 4.8	105.9 ± 4.9	0.89
Final LVM (g)	107.6 ± 3.8	103.9 ± 4.5	0.53
Change in LVM (g)	0.8 ± 3.2	-1.9 ± 2.8	0.52
Baseline LVMI (g/m^2)	55.5 ± 1.8	53.7 ± 2.0	0.52
Final LVMI (g/m^2)	55.6 ± 1.4	52.6 ± 1.9	0.21
Change in LVMI (g/m^2)	0.1 ± 1.5	-1.1 ± 1.2	0.53

Table 15 - Effect of allopurinol on LVM and LVMI

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: LVM = left ventricular mass; LVMI = left ventricular mass index.

3.5.4 Effect of Allopurinol on Left Ventricular volumes

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline LVESV (ml)	60.3 ± 3.3	56.2 ± 3.0	0.37
Final LVESV (ml)	61.6 ± 3.1	59.2 ± 3.4	0.61
Change in LVESV (ml)	1.3 ± 2.4	2.9 ± 2.6	0.63
Baseline LVESVI (ml/m^2)	31.4 ± 1.5	28.9 ± 1.5	0.24
Final LVESVI (ml/m^2)	31.7 ± 1.4	30.5 ± 1.7	0.57
Change in LVESVI (ml/m^2)	0.3 ± 1.2	1.6 ± 1.3	0.47
Baseline LVEDV (ml)	131.2 ± 5.	132.3 ± 5.5	0.88
Final LVEDV (ml)	138.4 ± 4.2	136.2 ± 5.5	0.74
Change in LVEDV (ml)	7.2 ± 4.4	3.8 ± 4.1	0.57
Baseline LVEDVI (ml/m^2)	68.5 ± 2.4	67.7 ± 2.5	0.81
Final LVEDVI (ml/m^2)	71.9 ± 2.0	69.6 ± 2.4	0.47
Change in LVEDVI (ml/m^2)	3.4 ± 2.1	1.9 ± 2.1	0.63

Table 16 - Effect of allopurinol on LVESV, LVESVI, LVEDV and LVEDVI

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: LVESV = left ventricular end-systolic volume; LVESVI = left ventricular end-systolic volume index; LVEDV = left ventricular end-diastolic volume; LVEDV = left ventricular end-diastolic volume index.

3.5.5 Effect of Allopurinol on Left Ventricular Stroke Volume & Ejection Fraction

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline LVSV (ml)	70.9 ± 3.4	76.3 ± 3.3	0.25
Final LVSV (ml)	76.7 ± 2.6	76.7 ± 3.4	0.99
Change in LVSV (ml)	5.8 ± 3.3	0.4 ± 2.6	0.20
Baseline LVSVI (ml/m^2)	37.1 ± 1.6	38.9 ± 1.4	0.40
Final LVSVI (ml/m^2)	40.0 ± 1.4	39.3 ± 1.4	0.72
Change in LVSVI (ml/m^2)	2.9 ± 1.7	0.3 ± 1.4	0.25
Baseline LVEF (%)	52.9 ± 1.9	56.8 ± 1.5	0.10
Final LVEF (%)	55.9 ± 1.6	56.8 ± 1.3	0.66
Change in LVEF (%)	3.0 ± 2.0	0.0 ± 1.3	0.20

Table 17 - Effect of allopurinol on LVSV, LVSVI and LVEF

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: LVSV = left ventricular stroke volume; LVSVI = left ventricular stroke volume index; LVEF = left ventricular ejection fraction.

3.5.6 Effect of Allopurinol on pulmonary haemodynamics

When analysing only complete cases and those who could have pulmonary haemodynamics measured at baseline visit, there were no significant difference in the change of pulmonary haemodynamic measurements between allopurinol group and placebo group.

Although there was better improvement in pulmonary acceleration time (PAT) in the allopurinol group, the change in PAT was not statistically significant within group (9.9, CI -21.0 to 1.2, p = 0.08).

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline Pulm_PWV (ms^{-1})	3.69 ± 0.34	3.16 ± 0.28	0.24
Final Pulm_PWV (ms^{-1})	3.48 ± 0.34	3.80 ± 0.37	0.53
Change in Pulm_PWV (ms^{-1})	-0.20 ± 0.45	0.64 ± 0.40	0.17
Baseline PAT (ms)	107.7 ± 3.1	104.8 ± 3.4	0.52
Final PAT (ms)	117.6 ± 3.8	106.7 ± 3.4	0.053
Change in PAT (ms)	9.9 ± 5.4	2.0 ± 5.5	0.31

Table 18 - Effect of allopurinol on pulmonary artery pulse wave velocity & PAT

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables.

Abbreviations: Pulm_PWV = pulmonary artery pulse wave velocity; PAT = pulmonary acceleration time.

3.6 Effect of Allopurinol on St George Respiratory Questionnaire

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline Total Score	47.2 ± 3.4	48.9 ± 2.9	0.71
Final Total Score	46.3 ± 3.3	47.6 ± 2.7	0.77
Change in Total Score	-0.9 ± 1.7	-1.3 ± 2.0	0.88
Baseline Symptom Score	47.1 ± 3.9	51.1 ± 3.9	0.47
Final Symptom Score	44.3 ± 4.3	52.7 ± 3.9	0.15
Change in Symptom Score	-2.8 ± 3.0	1.6 ± 3.7	0.35
Baseline Activity Score	65.5 ± 4.0	67.6 ± 3.6	0.69
Final Activity Score	67.4 ± 4.1	66.3 ± 3.3	0.84
Change in Activity Score	1.9 ± 2.3	-1.3 ± 2.4	0.29
Baseline Impact Score	36.9 ± 3.6	37.5 ± 3.0	0.89
Final Impact Score	35.2 ± 3.2	35.1 ± 2.7	0.99
Change in Impact Score	-1.7 ± 2.3	-2.4 ± 2.4	0.84

Table 19 - Mean change in SGRQ scores

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

3.7 Effect of Allopurinol on Short Form 36

	Allopurinol (n = 36)	Placebo (n = 35)	p
Physical functioning			
Baseline	39.4 ± 4.7	33.7 ± 4.4	0.37
Final	38.4 ± 4.6	36.6 ± 3.9	0.77
Change	-1.1 ± 3.1	2.9 ± 2.4	0.31
Physical role limitations			
Baseline	35.4 ± 6.3	22.1 ± 5.4	0.11
Final	37.1 ± 6.3	30.3 ± 5.9	0.44
Change	1.7 ± 5.7	8.2 ± 6.4	0.45
Emotional role limitations			
Baseline	61.1 ± 7.5	46.7 ± 6.9	0.15
Final	55.3 ± 7.2	48.1 ± 7.6	0.49
Change	-5.8 ± 6.5	1.5 ± 8.5	0.50
Energy or fatigue			
Baseline	47.1 ± 3.4	45.1 ± 3.0	0.67
Final	46.3 ± 3.4	49.7 ± 3.2	0.47
Change	-0.8 ± 2.6	4.5 ± 3.8	0.25
Emotional well-being			
Baseline	70.1 ± 3.2	73.4 ± 2.7	0.44
Final	74.8 ± 2.3	73.7 ± 3.0	0.77
Change	4.6 ± 2.8	0.3 ± 3.1	0.30
Social functioning			

Baseline	71.2 ± 4.1	71.4 ± 4.4	0.97
Final	68.8 ± 4.5	69.8 ± 4.3	0.87
Change	-2.4 ± 3.6	-1.6 ± 4.0	0.88
Pain			
Baseline	64.8 ± 4.9	62.6 ± 5.0	0.75
Final	60.7 ± 4.8	63.7 ± 4.7	0.66
Change	-4.1 ± 3.5	1.1 ± 4.7	0.37
General health			
Baseline	43.1 ± 3.4	41.9 ± 3.5	0.81
Final	43.2 ± 3.1	41.8 ± 2.6	0.74
Change	0.1 ± 2.7	-0.0 ± 3.3	0.97
Health change			
Baseline	47.9 ± 3.5	39.3 ± 4.0	0.10
Final	53.5 ± 2.5	46.2 ± 2.9	0.06
Change	5.6 ± 4.0	7.0 ± 5.3	0.83

Table 20 - Mean change in SF-36 scores

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

3.8 Effect of Allopurinol on King's Brief Interstitial Lung Disease questionnaire

Five patients (3 in placebo and 2 in allopurinol) with ILD completed the King's Brief Interstitial Lung Disease (K-BILD) questionnaire at baseline but only three completed the study (2 in placebo and 1 in allopurinol). Completed cases analysis was done. At baseline, the patient in the allopurinol group had higher scores in all the four different domains (psychological score, breathless score, breathlessness and activities score, chest symptom score, and total score) compared to the two patients in the placebo group. An improvement in all four domains was observed in the two patients in the placebo group compared to the one patient in allopurinol group whose scores all decreased after 12 months of treatment.

3.9 Effect of Allopurinol on BDI-TDI

When analysing completed cases who had both BDI and TDI measured (n = 54), there was a significant difference in the TDI score (p = 0.02). 9 of the total completed cases were not analysed as these participants indicated that it was not breathlessness which was the underlying cause for their limitation in exercise capacity.

	Allopurinol (n = 28)	**Placebo (n = 26)**	**p**
BDI	5.5 ± 1.7	5.4 ± 2.0	0.88
TDI	-0.7 ± 0.9	-0.1 ± 0.9	0.02

Table 21 - Effect of allopurinol on BDI-TDI score

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: BDI = baseline dyspnea index; TDI = transition dyspnea index

3.10 Effect of Allopurinol on spirometry

The baseline spirometric measurements were similar in both allopurinol and placebo groups. The spirometric measurements include FEV_1, FVC and FEF_{25-75}. Allopurinol did not produce any significant change in the spirometric measurements after 12 months of treatment. There were also no significant differences in the change in spirometric measurements between the two groups after 12 months.

3.11 Effect of Allopurinol on 6-minute walk test

The total walking distance in metres, the pre-test O_2, the post-test O_2, the pre-test HR, the post-test HR, the pre-test Borg Dyspnoea score, the post-test Borg Dyspnoea score, the pre-test Borg Fatigue score and the post-test Borg Fatigue score were measured for each 6MWT. Missing values were calculated using multiple imputations. The mean change in the parameters were then calculated, comparing those on allopurinol with those on placebo.

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline distance	355.3 ± 17.4	375.6 ± 16.5	0.40
Final distance	364.1 ± 15.1	365.6 ± 16.7	0.95
Change in distance	8.8 ± 10.0	-10.0 ± 12.5	0.24
Baseline pre-test O_2	95.8 ± 0.5	95.5 ± 0.5	0.67
Final pre-test O_2	95.4 ± 0.4	94.8 ± 0.7	0.39
Change in pre-test O_2	-0.4 ± 0.3	-0.8 ± 0.4	0.51
Baseline post-test O_2	91.9 ± 1.3	90.8 ± 1.1	0.52
Final post-test O_2	90.6 ± 0.8	90.2 ± 1.1	0.79
Change in post-test O_2	-1.3 ± 0.9	-0.5 ± 0.8	0.52
Baseline pre-test HR	80.0 ± 2.3	82.3 ± 3.1	0.54
Final pre-test HR	78.8 ± 2.3	79.2 ± 2.5	0.90
Change in pre-test HR	-1.2 ± 2.3	-3.1 ± 3.2	0.62

Baseline post-test HR	94.1 ± 2.8	102.9 ± 4.4	0.88
Final post-test HR	97.9 ± 2.6	100.2 ± 3.3	0.57
Change in post-test HR	3.8 ± 2.1	-2.6 ± 3.6	0.12
Baseline pre-test Dyspnoea Score	0.8 ± 0.2	0.8 ± 0.2	0.99
Final pre-test Dyspnoea Score	1.1 ± 0.2	1.0 ± 0.2	0.86
Change in pre-test Dyspnoea Score	0.3 ± 0.2	0.3 ± 0.2	0.86
Baseline post-test Dyspnoea Score	3.4 ± 0.4	3.0 ± 0.3	0.37
Final post-test Dyspnoea Score	3.4 ± 0.4	3.8 ± 0.5	0.48
Change in post-test Dyspnoea Score	0.0 ± 0.4	0.9 ± 0.5	0.19

Table 22 - Mean change in 6MWT measurements

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: 6MWT = six minute walk test; O_2 = oxygen saturation; HR = heart rate

3.12 Effect of Allopurinol on blood markers

The blood markers that were measured at baseline and at final visits were NT-proBNP, hs-Trop I and urate levels. Allopurinol did not significantly reduced NT-proBNP and hs-Trop I level compared with placebo. Allopurinol significantly reduced serum uric acid (allopurinol group -205.9 ± 14.9 vs. placebo group -4.6 ± 8.5; $p < 0.001$). The serum uric acid level was reduced by 59% with allopurinol treatment.

	Allopurinol (n = 36)	**Placebo (n = 35)**	**p**
Baseline NT-proBNP (pg/ml)	916.81 ± 248.62	949.31 ± 275.13	0.93
Final NT-proBNP (pg/ml)	829.18 ± 233.20	1238.68 ± 376.36	0.35
Change in NT-proBNP (pg/ml)	-87.6 ± 127.2	289.4 ± 213.3	0.13
Baseline hs-Trop I (pg/ml)	3.22 ± 0.55	3.59 ± 0.73	0.69
Final hs-Trop I (pg/ml)	3.03 ± 0.41	3.77 ± 0.79	0.40
Change in hs-Trop I (pg/ml)	-0.20 ± 0.48	0.18 ± 0.37	0.53
Baseline Urate (µmol/l)	350.06 ± 13.54	342.94 ± 15.37	0.73

Final Urate (μmol/l)	144.12 ± 9.12	338.34 ± 15.59	< 0.001
Change in Urate (μmol/l)	-205.9 ± 14.9	-4.6 ± 8.5	< 0.001

Table 23 - Change in blood markers

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: NT-proBNP = N-terminal prohormone of brain natriuretic peptide; hs-Trop I = high sensitivity troponin I.

3.13 Subgroup analysis

To look whether any subgroup might have benefitted from allopurinol, the participants were stratified by high/low baseline RVM, high/low baseline RVMI, high/low baseline serum urate level, high/low baseline pulmonary acceleration time (measured by doppler echocardiography), high/low baseline NT-proBNP level and high/low baseline six-minute walk distance using the median values of the corresponding parameter, and by COPD GOLD stage. The study was not powered to evaluate subgroups, but these post-hoc subgroup analyses were performed to help demonstrate that the data has been fully explored.

3.13.1 Subgroup analysis stratified by median of RVM

The median for baseline right ventricular mass (RVM) is 39.56g.

	Allopurinol (n = 18)	**Placebo (n = 16)**	**p**
Baseline RVM (g)	47.73 ± 1.35	50.69 ± 2.55	NS
Final RVM (g)	46.56 ± 2.01	49.40 ± 3.04	NS
Change in RVM (g)	-1.17 ± 1.67	-1.29 ± 1.49	0.96
Baseline RVMI (g/m^2)	23.38 ± 0.70	24.47 ± 1.09	NS
Final RVMI (g/m^2)	22.94 ± 0.82	24.04 ± 1.40	NS
Change in RVMI (g/m^2)	-0.44 ± 0.83	-0.44 ± 0.71	0.997

Table 24 - Effect of allopurinol on RVM and RVMI for participants with RVM > 39.56 g

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

	Allopurinol (n = 13)	Placebo (n = 15)	p
Baseline RVM (g)	35.00 ± 1.44	33.19 ± 1.29	NS
Final RVM (g)	35.45 ± 1.19	35.01 ± 1.85	NS
Change in RVM (g)	-0.85 ± 1.25	1.82 ± 1.15	0.13
Baseline RVMI (g/m^2)	19.63 ± 0.73	17.88 ± 0.44	NS
Final RVMI (g/m^2)	19.84 ± 0.65	18.93 ± 0.63	NS
Change in RVMI (g/m^2)	-0.38 ± 0.65	1.05 ± 0.61	0.12

Table 25 - The effect of allopurinol on RVM and RVMI for participants with RVM < 39.56 g

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index

3.13.2 Subgroup analysis stratified by median of RVMI

The median for baseline right ventricular mass index (RVMI) is 20.72 g/m^2.

	Allopurinol (n = 19)	**Placebo (n = 14)**	**p**
Baseline RVM (g)	46.07 ± 1.63	51.29 ± 2.89	NS
Final RVM (g)	42.77 ± 2.24	50.05 ± 3.43	NS
Change in RVM (g)	-3.30 ± 1.29	-1.24 ± 1.70	0.33
Baseline RVMI (g/m^2)	23.88 ± 0.50	25.29 ± 1.06	NS
Final RVMI (g/m^2)	22.33 ± 0.85	25.00 ± 1.43	NS
Change in RVMI (g/m^2)	-1.55 ± 0.65	-0.30 ± 0.82	0.24

Table 26- Effect of allopurinol on RVM and RVMI for participants with RVMI > 20.72 g/m^2

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

	Allopurinol (n = 11)	Placebo (n = 18)	p
Baseline RVM (g)	38.12 ± 1.37	34.94 ± 1.48	NS
Final RVM (g)	40.98 ± 2.29	35.90 ± 1.75	NS
Change in RVM (g)	2.86 ± 1.42	0.96 ± 1.09	0.30
Baseline RVMI (g/m^2)	19.08 ± 0.24	18.11 ± 0.41	NS
Final RVMI (g/m^2)	20.62 ± 0.77	18.62 ± 0.54	NS
Change in RVMI (g/m^2)	1.54 ± 0.70	0.51 ± 0.58	0.24

Table 27 - Effect of allopurinol on RVM and RVMI for participants with RVMI < 20.72 g/m^2

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index

3.13.3 Subgroup analysis stratified by median of urate level

The median for serum urate level is 334 μmol/l.

	Allopurinol (n = 18)	**Placebo (n = 13)**	**p**
Baseline RVM (g)	44.03 ± 1.97	47.86 ± 3.64	NS
Final RVM (g)	44.34 ± 1.95	48.21 ± 3.96	NS
Change in RVM (g)	0.31 ± 1.44	0.36 ± 2.12	0.99
Baseline RVMI (g/m^2)	21.71 ± 0.72	22.91 ± 1.66	NS
Final RVMI (g/m^2)	22.10 0.72	23.34 ± 1.87	NS
Change in RVMI (g/m^2)	0.39 ± 0.71	0.43 ± 0.97	0.97

Table 28 - Effect of allopurinol on RVM and RVMI for participants with urate > 334 μmol/l

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

There were significant differences in the change in RVM and RVMI between allopurinol and placebo groups in the participants with low baseline urate level (< 334 µmol/l). However, these differences are statistically significant because there was already significant difference in baseline RVM and RVMI between both groups.(*Table 29*)

	Allopurinol (n = 12)	**Placebo (n = 18)**	**p**
Baseline RVM (g)	41.84 ± 1.59	38.15 ± 2.22	0.030
Final RVM (g)	38.77 ± 2.65	38.27 ± 2.10	NS
Change in RVM (g)	-3.07 ± 1.60	0.12 ± 0.77	0.009
Baseline RVMI (g/m^2)	22.74 ± 0.80	20.11 ± 0.75	0.081
Final RVMI (g/m^2)	21.10 ± 1.12	20.29 ± 0.68	NS
Change in RVMI (g/m^2)	-1.64 ± 0.79	0.18 ± 0.47	0.010

Table 29 - Effect of allopurinol on RVM and RVMI for participants with urate < 334 µmol/l

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

3.13.4 Subgroup analysis stratified by median of pulmonary acceleration time

The median for pulmonary acceleration time (PAT) is 97 ms.

	Allopurinol (n = 15)	**Placebo (n = 16)**	**p**
Baseline RVM (g)	42.49 ± 1.84	36.75 ± 1.71	0.030
Final RVM (g)	43.03 ± 2.39	37.21 ± 1.82	NS
Change in RVM (g)	0.54 ± 1.67	0.45 ± 1.15	0.97
Baseline RVMI (g/m^2)	21.47 ± 0.68	19.65 ± 0.74	NS
Final RVMI (g/m^2)	21.72 ± 0.86	20.17 ± 0.70	NS
Change in RVMI (g/m^2)	0.25 ± 0.82	0.52 ± 0.59	0.79

Table 30 - Effect of allopurinol on RVM and RVMI for participants with PAT > 97 ms

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

	Allopurinol (n = 15)	Placebo (n = 15)	p
Baseline RVM (g)	43.82 ± 1.99	48.06 ± 3.51	NS
Final RVM (g)	41.20 ± 2.27	48.02 ± 3.68	NS
Change in RVM (g)	-2.62 ± 1.37	-0.04 ± 1.64	0.24
Baseline RVMI (g/m^2)	22.77 ± 0.82	23.02 ± 1.46	NS
Final RVMI (g/m^2)	21.69 ± 0.92	23.06 ± 1.65	NS
Change in RVMI (g/m^2)	-1.08 ± 0.73	0.04 ± 0.79	0.31

Table 31 - Effect of allopurinol on RVM and RVMI for participants with PAT > 97 ms

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

3.13.5 Subgroup analysis stratified by median of NT-proBNP level

The median for NT-proBNP level is 489.53 pg/ml.

	Allopurinol (n = 13)	**Placebo (n = 18)**	**p**
Baseline RVM (g)	40.52 ± 1.99	44.47 ± 3.08	NS
Final RVM (g)	41.56 ± 2.17	43.91 ± 3.38	NS
Change in RVM (g)	1.04 ± 1.33	-0.56 ± 4.42	0.44
Baseline RVMI (g/m^2)	20.93 ± 0.96	22.62 ± 1.22	NS
Final RVMI (g/m^2)	21.56 ± 0.83	22.57 ± 1.43	NS
Change in RVMI (g/m^2)	0.63 ± 0.72	-0.05 ± 0.67	0.50
Baseline LVEF	52.49 ± 3.00	56.82 ± 3.00	NS
Final LVEF	57.61 ± 2.34	55.20 ± 2.26	NS
Change in LVEF	5.12 ± 2.36	-1.62 ± 1.51	0.017

Table 32 - Effect of allopurinol on RVM, RVMI and LVEF for participants with NT-proBNP > 489.53 pg/ml

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index, LVEF = left ventricular ejection fraction

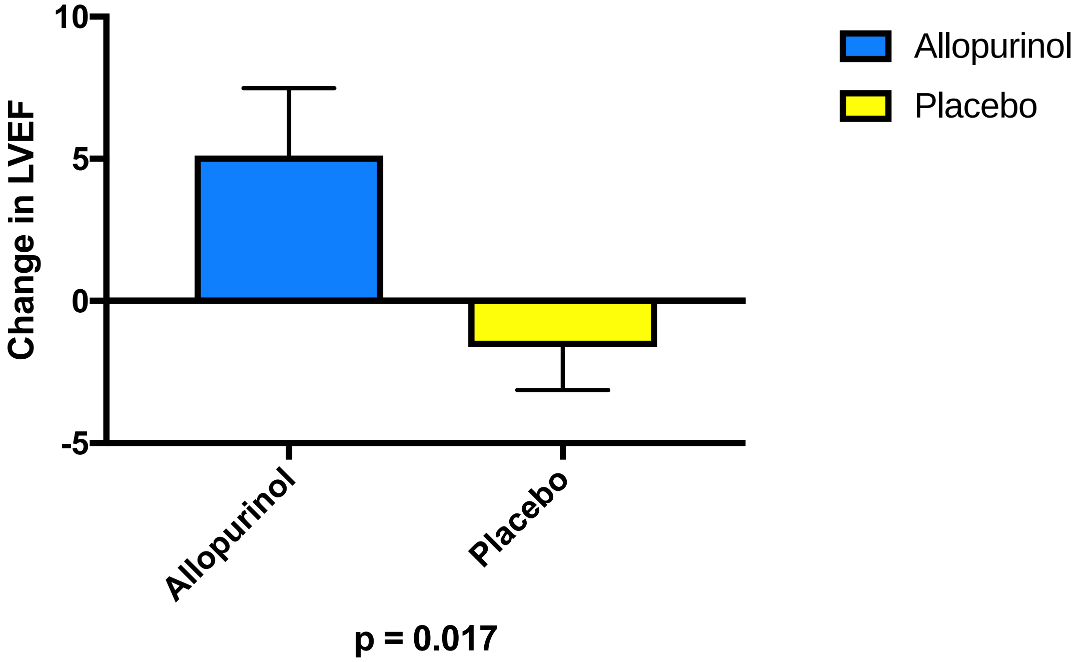

Figure 24 - Change in LVEF for participants with high NT-proBNP

There were no significant differences in the change from baseline in the other RV and LV parameters when comparing allopurinol group with placebo group, in the high NT-proBNP population.

	Allopurinol (n = 17)	Placebo (n = 14)	p
Baseline RVM (g)	45.17 ± 1.69	39.03 ± 2.52	0.047
Final RVM (g)	42.54 ± 2.40	39.74 ± 2.39	NS
Change in RVM (g)	-2.62 ± 1.58	0.71 ± 1.28	0.12
Baseline RVMI (g/m^2)	23.03 ± 0.53	19.49 ± 0.84	0.001
Final RVMI (g/m^2)	21.81 ± 0.90	19.91 ± 0.73	NS
Change in RVMI (g/m^2)	-1.22 ± 0.77	0.42 ± 0.72	0.14
Baseline LVEF	55.46 ± 1.29	59.07 ± 2.10	NS
Final LVEF	54.58 ± 2.75	59.10 ± 1.65	NS
Change in LVEF	-0.87 ± 2.02	0.03 ± 1.56	0.73

Table 33 - Effect of allopurinol on RVM, RVMI and LVEF for participants with NT-proBNP < 489.53 pg/ml

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index, LVEF = left ventricular ejection fraction.

3.13.6 Subgroup analysis stratified by median of 6MWD

The median for six-minute walk distance (6MWD) is 384.5 m.

	Allopurinol (n = 13)	**Placebo (n = 18)**	**p**
Baseline RVM (g)	40.88 ± 2.07	43.65 ± 2.46	NS
Final RVMI (g)	39.86 ± 1.77	43.46 ± 2.32	NS
Change in RVMI (g)	-1.02 ± 1.82	-0.19 ± 1.29	0.71
Baseline RVM (g/m^2)	21.03 ± 0.53	21.37 ± 1.05	NS
Final RVMI (g/m^2)	20.75 ± 0.63	21.49 ± 1.03	NS
Change in RVMI (g/m^2)	-0.27 ± 0.89	0.13 ± 0.60	0.70

Table 34 - Effect of allopurinol on RVM and RVMI for participants with 6MWD > 384.5 m

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

	Allopurinol (n = 17)	Placebo (n = 13)	p
Baseline RVM (g)	44.89 ± 1.68	40.24 ± 3.87	NS
Final RVM (g)	43.84 ± 2.50	41.02 ± 4.28	NS
Change in RVM (g)	-1.06 ± 1.40	0.78 ± 1.53	0.39
Baseline RVMI (g/m^2)	22.96 ± 0.81	21.16 ± 1.45	NS
Final RVMI (g/m^2)	22.43 ± 0.96	21.67 ± 1.67	NS
Change in RVMI (g/m^2)	-0.53 ± 0.72	0.50 ± 0.82	0.35

Table 35 - Effect of allopurinol on RVM and RVMI for participants with 6MWD < 384.5 m

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

3.13.7 Subgroup analysis stratified by FEV_1 GOLD Stage

	Allopurinol (n = 23)	**Placebo (n = 18)**	**p**
Baseline RVM (g)	42.87 ± 1.57	43.44 ± 2.71	NS
Final RVM (g)	43.39 ± 1.83	43.27 ± 2.42	NS
Change in RVM (g)	0.52 ± 1.13	-0.17 ± 1.17	0.68
Baseline RVMI (g/m^2)	21.53 ± 0.57	21.48 ± 1.06	NS
Final RVMI (g/m^2)	21.94 ± 0.68	21.58 ± 1.02	NS
Change in RVMI (g/m^2)	0.41 ± 0.57	0.10 ± 0.58	0.71

Table 36 - Effect of allopurinol on RVM and RVMI for participants with FEV_1 GOLD Stages 1 & 2

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

	Allopurinol (n = 7)	**Placebo (n = 13)**	**p**
Baseline RVM (g)	44.09 ± 2.72	40.54 ± 3.56	NS
Final RVM (g)	37.92 ± 3.27	41.29 ± 4.17	NS
Change in RVM (g)	-6.16 ± 1.97	0.75 ± 1.71	0.022
Baseline RVMI (g/m^2)	24.07 ± 1.15	21.00 ± 1.42	NS
Final RVMI (g/m^2)	20.93 ± 1.49	21.54 ± 1.67	NS
Change in RVMI (g/m^2)	-3.13 ± 1.00	0.54 ± 0.85	0.015

Table 37 - Effect of allopurinol on RVM and RVMI for participants with FEV_1 GOLD Stages 3 & 4

Data are mean ± SEM, unless otherwise indicated. Independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

Abbreviations: RVM = right ventricular mass; RVMI = right ventricular mass index.

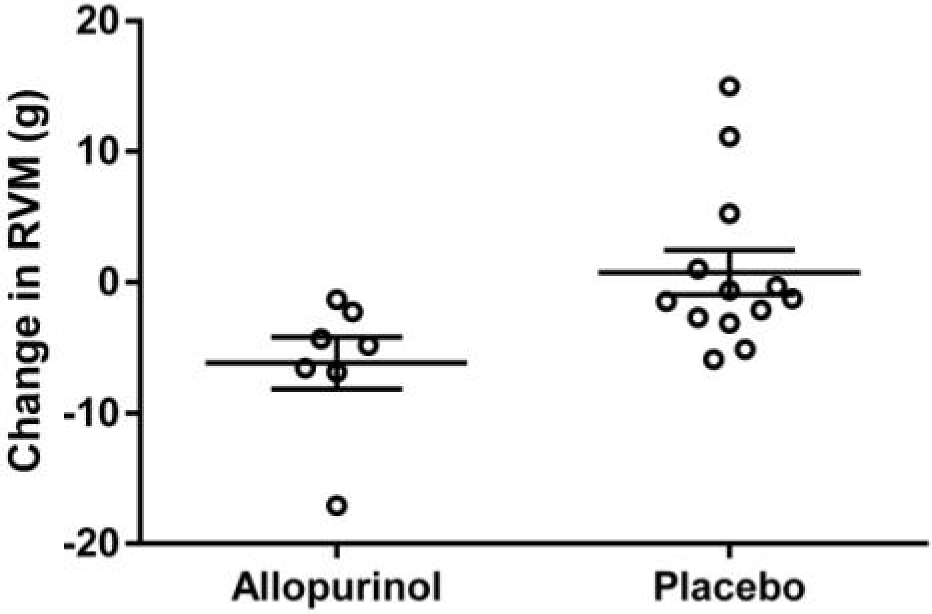

Figure 25 - Change in RVM for participants with GOLD 3 & 4

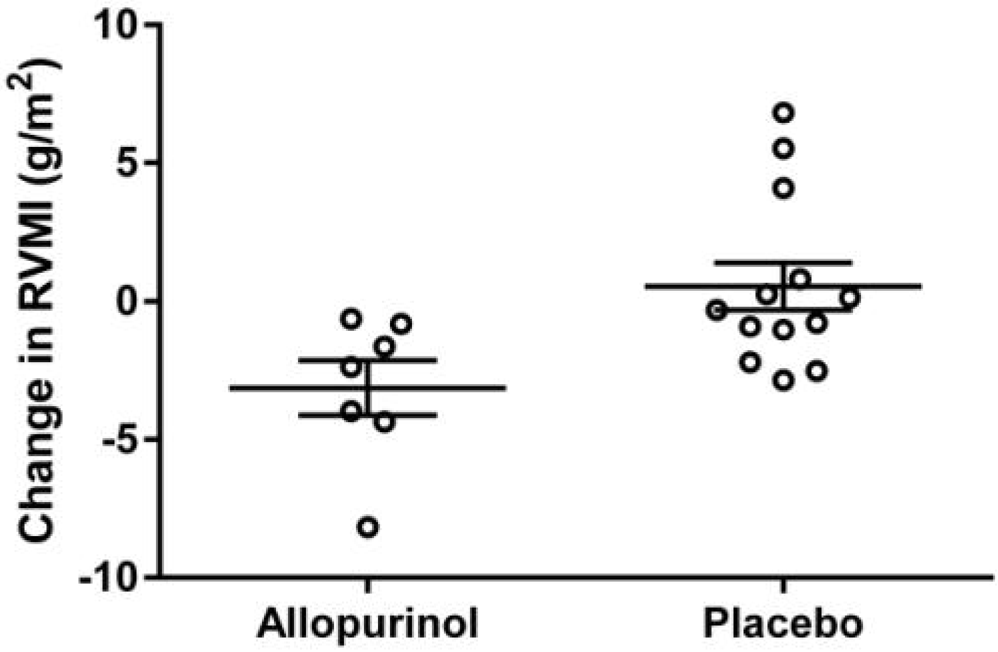

Figure 26 - Change in RVMI for participants with GOLD 3 & 4

3.14 Adverse Events (AE)

There was a total of 179 adverse events recorded:

- 99 AE's in placebo group
    - 5 AE's possible causality from IMP, all recovered
- 80 AE's in allopurinol group
    - 3 AE's possible causality from IMP from same participant (malaise x2, lethargy). All three adverse events recovered when IMP dose reduced.

There were 7 serious adverse events (SAE) in the allopurinol group:

- Infective exacerbation of COPD
- Pneumonia
- Left leg cellulitis
- Prostate cancer
- Acute encephalopathy – unclear cause, probable opiate intoxication
- Transient oesophageal obstruction
- Community acquired pneumonia

There were 15 SAE's in the placebo group:

- Dehydration and chest infection
- Bladder tumour
- Metastatic cancer
- Drug-induced syncope
- Non-specific abdominal pain

- Diverticular disease
- Fracture left neck of femur
- Rectal bleed due to diverticular disease
- Urinary tract infection
- Postural hypotension
- Rectus sheath haematoma
- Atrial flutter
- Syncope
- Death due to atherosclerosis
- Urinary retention

3.15 Study drug compliance

The change in serum uric acid levels reflect the study drug compliance. Allopurinol reduced serum uric acid level by 59% after 12 months of therapy whilst there was no significant change in the placebo arm. All the participants in the allopurinol group had a reduction in uric acid levels, suggesting that they were compliant in taking the study drug.

In addition, the number of tablets returned by each participant was counted at each study visit. All the participants included in the study analysis had a compliance of more than 70%. The overall average drug compliance was 96% from tablet count.

4. Discussion

In summary, this randomised, double-blind, placebo-controlled, parallel-group study aimed to evaluate whether treatment with high dose allopurinol would regress right ventricular hypertrophy in patients with pulmonary hypertension associated with chronic lung disease (i.e chronic obstructive pulmonary disease, interstitial lung disease). In this section, the findings of the study will be discussed and the possible underlying mechanism for these findings. The strengths and the limitations of this study will also be considered.

4.1 Primary outcome: Right Ventricular Mass & Right Ventricular Mass Index

The study was powered to detect a 5 g change in right ventricular mass (RVM). Despite having a similar baseline right ventricular mass in both allopurinol and placebo groups, there was no significant difference in the change in RVM between the two groups (allopurinol 1.85 g vs placebo 0.97 g) using intention-to-treat analysis. The complete-cases analysis resulted in similar finding. The possible reasons for why the overall study result was negative are the lack of effect of allopurinol on the right ventricle and the heterogeneity of this current study population. These are discussed in more details subsequently.

The mechanism underlying right ventricular hypertrophy (RVH) may be different from left ventricular hypertrophy (LVH). Most studies investigating cardiac hypertrophy[120,121] mainly looked at the left ventricle. Many of the findings from these studies are then translated from LVH to RVH. The current study did the same as it was based on findings from previous studies[151,173,174] where allopurinol was found to regress LVH. In addition, Rekhraj et al.[173] demonstrated in their study that allopurinol reduced LV end systolic volume and LV afterload, suggesting that offloading the LV may have been the mechanism for the LVH regression. Therefore, the underlying mechanism for right ventricular hypertrophy could involve more than oxidative stress and mechanical stretch due to increased afterload pressure. Allopurinol could still have had an effect on the right ventricle by decreasing oxidative stress which is directly implicated in ventricular hypertrophy. However, the effect from allopurinol may have been too small to be demonstrated in this study.

A second explanation is that the participants who were enrolled in the study were heterogeneous in terms of the severity of their chronic lung disease. Recent studies[21] have demonstrated the wide range of phenotypes and heterogeneity of patients suffering from COPD. Hence the overall effect of allopurinol on right ventricular hypertrophy could have been different if we had considered only patients with specific phenotypes of the disease. As it was a proof of concept study, it was difficult to restrict to specific phenotypes as there was no previous studies that had already evaluated this

As described above, the heterogenous study population may have masked a possible effect of allopurinol. This is supported by the finding that there was a statistically significant difference in the allopurinol group (-6.16 g) compared to the placebo group (0.75 g) when complete-case analysis was performed in a subgroup of patients with more severe airflow obstruction (i.e patients with COPD GOLD 3 and 4). This positive finding suggests that the worse the lungs are, the more likely allopurinol will regress right ventricular hypertrophy. A potential explanation for the positive finding in more severe airflow limitation is that these patients are more likely to be more hypoxic on exertion (despite having satisfactory resting oxygen saturation), causing increasing oxidative stress, leading to cardiac hypertrophy and increased pulmonary arterial pressures on exercise. However, this result should be interpreted with caution as the study was not powered to investigate this subgroup and the number of patients in this subgroup (n = 20) was relatively small. On the other hand, this could still be a signal that allopurinol could have a beneficial effect in patients with more severe airflow obstruction and future trials studying this population could unmask this effect.

Another possible explanation for the overall negative result for the primary outcome is that the study population consisted mostly of patients with chronic obstructive pulmonary disease (93%) and a few patients suffering from interstitial lung disease (7%). It was agreed at the time when the study was designed to include only patients suffering from group 3 pulmonary hypertension. However, it is still not completely clear whether the pathophysiology underlying the development of pulmonary hypertension and right ventricular hypertrophy is the same for all the chronic lung diseases.

The right ventricular mass of this study population was relatively not too high compared to studies in patients with idiopathic pulmonary arterial hypertension[203,205-209]. This is probably because pulmonary hypertension in COPD and ILD patients tends to be milder in severity compared to group 1 patients.[1] There is reason to think that with bigger RVH, the effect of allopurinol would be more appreciable. However, this was not demonstrated in this current study as despite stratifying by high/low baseline RVM or RVMI, there was no significant difference in the change in RVM and in RVMI in the subgroup of participants with high baseline RVM or RVMI.

It is difficult to compare the results of the current study with other randomised clinical trial (RCT) as to this date there is no other RCT which has been done looking at the cardiac mass, volume and function in patients with group 3 pulmonary hypertension. The closest studies that could be compared with are longitudinal studies in patients with group 1 pulmonary hypertension who received drug therapies already licensed to treat pulmonary hypertension in this group. The results from these studies were mixed: the studies by

Michelakis et al.[207], van Wolferen et al.[205] and van de Veerdonk[209], and the SERAPH study[203] have reported a reduction in RV mass from baseline while the study by Roeleveld et al.[208] and the EURO-MR study[206] reported no change in RVM.

To look if there was any other subgroup that might have benefitted from allopurinol, the participants were also stratified by high/low baseline serum urate level, high/low baseline pulmonary acceleration time (measured by doppler echocardiography), high/low baseline NT-proBNP level and high/low baseline six-minute walk distance, using the median values of the corresponding parameter. None of these subgroups made any changes to the results of change in RVM and in RVMI. The study was not powered to evaluate subgroups, but these subgroup analyses were included to help demonstrate that the data has been fully explored.

4.2 Right ventricular mass as primary outcome measure

There were several reasons to have RV as the primary outcome. Allopurinol was likely to exert benefits on RVM which are not due to the pulmonary vasculature. The pathophysiology of the right ventricle in lung disease is more complex than pulmonary hypertension simply increasing RV afterload and RV mass, as exemplified by the fact that RVH often occurs in COPD before pulmonary vascular abnormalities.[89] This is almost certainly because oxidative stress in lung disease independently causes both RVH and pulmonary vascular abnormalities. There is experimental evidence demonstrating a strong direct effect of OS promoting ventricular hypertrophy.[120] Since allopurinol profoundly reduces OS, it is likely to reduce RV structure and function by a direct effect on the RV before it also reduces RVH by causing pulmonary vasodilatation.

Since RVH is the initiating step in the progression to right ventricular failure in lung disease, its reversal is a key surrogate and allopurinol was likely to do this both directly (on the RV) and indirectly by acting on the pulmonary vasculature. This dual effect emphasies why RVH is the best surrogate by which to judge allopurinol rather than the pulmonary vasculature as there are likely to be RVH effects of allopurinol which are not due to pulmonary vascular effects (and are due instead to altering OS). Therefore, in the unlikely event that allopurinol does not alter the pulmonary vasculature, it could still benefit the RV (by reducing OS), which is why RVH was the primary.

Furthermore, since RVH leads to RV dysfunction, it seems better to tackle the initiating event rather than trying to target a downstream event like RV dysfunction which could be harder to alter. Overall, RVM is the pivotal endpoint in this proof of concept study as it is the central link between OS, and later RV dysfunction.

4.3 Secondary outcomes

4.3.1 Other cardiac MRI measurements

As the non-invasive cardiac MRI scan provides a comprehensive picture of the right ventricular structure and function[206], right ventricular volumes and function were also measured. Right ventricular end-diastolic volume (RVEDV), end-systolic volume (RVESV), stroke volume (RVSV) and ejection fraction (RVEF) were measured at baseline and after 12-month of treatment. There were no significant differences in the baseline right ventricular volumes and function. After twelve months of treatment, there were no significant differences in the change in RVEDV, RVESV, RVSV, RVEF and their index values.

The left ventricular volumes (LVEDV, LVESV, LVSV) and ejection fraction (LVEF) were also measured at baseline and at final visit. Despite similar baseline measurements in both groups (allopurinol and placebo), there were no significant differences in the change in LVEDV, LVESV, LVSV, LVEF and their index values between the two groups.

Improvements in left ventricular volumes were reported in other studies with allopurinol. Kao *et al.*[151] have demonstrated that allopurinol tended to cause a fall in LVEDV in patients with chronic kidney disease (allopurinol -9.64 ml vs placebo -1.65 ml, p = 0.084). Rekhraj *et al.*[173] have reported a significant reduction in LVESV in allopurinol (-2.18 ml) compared to placebo (+1.3 ml) in patients with ischaemic heart disease. The absence of change in ventricular volumes in the current study could possibly be because the underlying pathophysiological process of pulmonary hypertension affecting the heart is different compared with chronic kidney disease and ischaemic heart disease.

Two recent studies[210,211] have reported improvements in ventricular volumes and function in COPD population when treatment with inhaled therapy. However, these studies were looking at a particular phenotype of COPD population, namely with lung hyperinflation. There was no such emphasis in the current study. There was also no formal assessment lung hyperinflation in the study which is usually done by body plethysmography. In addition, the absence of change in ventricular volumes adds more evidence that allopurinol did not have any effect on lung volumes.

Subgroup analyses for other CMRI measurements were done like the ones done for the primary outcome. The subgroup analysis when stratifying by high/low NT-proBNP level has shown some promising results. The patients (n = 31) with higher NT-proBNP level (> 489 pg/ml) had a greater improvement in LVEF with allopurinol +5.12% vs placebo -1.62% (mean diff 7.33, CI 1.88 to 12.78, p = 0.02). A possible mechanism for this positive finding is that allopurinol could be improving cardiac energetics by improving relative and absolute concentrations of myocardial high-energy phosphates and ATP flux through creatinine kinase, which has already been observed in left heart failure[160,161].

4.3.2 Six-minute walk distance

There was non-significant difference in the baseline six-minute walk distance (6MWD) between the allopurinol group (355.3 m) and the placebo group (375.6 m). After 12 months of treatment, a slight increase in 6MWD was observed in the allopurinol group (8.8 m) compared to a decrease in the placebo group (-10.0 m). The difference in the change in 6MWD between the two groups was not statistically significant.

The improvement in 6MWD in the allopurinol group did not reach the minimal clinically important difference (MCID) of 33 m which is usually quoted in studies for pulmonary hypertension. The worsening in 6MWD in the placebo group was likely due to the progression of the underlying chronic lung disease with associated deconditioning. Hence, these findings may suggest that allopurinol might have had an effect by decreasing the rate of deterioration in 6MWD. However, as the improvement in 6MWD in the allopurinol group was not statistically significant and did not reach MCID, it is difficult to be sure about this beneficial effect of allopurinol. Further studies with a bigger population looking at 6MWD as primary outcome would help to evaluate this further.

6MWD does not solely reflect cardiac function and can be affected by factors such as motivation, mood, co-morbidities, patient’s age, height, weight and muscular training.[7] Hence any improvement in 6MWD does not automatically imply that allopurinol improved 6MWD by improving cardiac function.

In addition, it was noted that allopurinol did not affect the pre-test and post-test oxygen saturation, heart rate, Borg dyspnoea score and Borg fatigue score.

Other exercise tests have been used to measure exercise capacity in various medical conditions. These include incremental exercise tests such as cycling exercise test (CPET) and incremental shuttle walk test (ISWT), and endurance exercise tests such as cycle endurance test (CET) and endurance shuttle walk test (ESWT). Mainguy *et al.* have demonstrated in their study that the four exercise tests described above (CPET, ISWT, CET and ESWT) all have some correlation with the pulmonary pressures but using them as an alternative to 6MWT for tracking beneficial clinical changes following therapy remained to be confirmed.[212] A previous study from the same group showed that 6MWT had the best ability, compared with CET and ESWT, to capture changes in exercise capacity and less variation between repeated measures when sildenafil was added to patient's baseline monotherapy.[213]

Actigraphy is a promising novel tool that assesses 24-hour activity patterns of ambulatory patients (reflecting the patient's activity during daily life at home). It has the potential to serve as a prognostic and outcome parameter in practice and in scientific trials. Ulrich *et al.* used wrist actigraphy in their study which demonstrated that patients (diagnosed with PAH or CTEPH) with a duration of daily activity of less than 15 hours had a significant shorter survival without lung transplantation compared to patients who were more active.[214] The usefulness of actigraphy needs to be confirmed in larger cohorts. In addition, a direct comparison between actigraphy and 6MWT is also required to see if it could be used as an alternative to 6MWT in the assessment of exercise capacity following treatment.

4.3.3 Blood markers

NT-proBNP is a marker of disease progression in patients with pulmonary hypertension. The ESC/ERS guidelines[1] advocate using serial measurements of NT-proBNP to monitor disease progression and to assess treatment effects.

The baseline NT-proBNP levels were similar in both groups (allopurinol 916.81 pg/ml vs placebo 949.31 pg/ml). These baseline values were above the 300 pg/ml threshold which put the study population in the intermediate-severe risk category as defined by the ESC guidelines[1].

The NT-proBNP level was increased in the placebo group (+289.4 pg/ml) compared to a slight reduction in the allopurinol group (-87.6 pg/ml) after 12 months. Although the difference in the change in NT-proBNP levels was not significant between the two groups, this may suggest that there was possibly a worsening of the heart function with time in the placebo group and that allopurinol may have some beneficial effects on the heart. It is difficult to prove this theory as there was no significant change in cardiac measurements done in this study after 12 months of treatment. High plasma levels and in particular, a further increase in plasma BNP have a strong independent association with mortality.[7] Hence, if allopurinol does reduce plasma BNP levels, this may confer a survival benefit.

Even in the subgroup of more severe airflow limitation (GOLD group 3 & 4), the significant difference noted in the change in RVM or RVMI in the allopurinol group compared to the placebo group was not accompanied by a significance difference in the change in NT-proBNP level between the two groups.

It is probably safer to say that allopurinol did not have any effects on NT-proBNP levels in patients with pulmonary hypertension associated with chronic lung disease. This would be in line with the results of previous studies[173,174] where a significant change in ventricular mass was observed without a change in NT-proBNP level.

Cardiac troponins are cardiac biomarkers. Concentrations of cardiac troponins do not increase unless myocyte damage has occurred. High-sensitivity cardiac troponins are powerful predictors of long-term mortality and cardiovascular disease in the general population.[215]

The baseline levels of high-sensitive troponin I (hs-Trop I) were similar in both allopurinol (3.22 pg/ml) and placebo (3.59 pg/ml) groups. The level of hs-Trop I increased in the placebo group (+0.18 pg/ml) and slightly reduced in the allopurinol group (-0.20 pg/ml). The difference in the change in hs-Trop I levels was not significant. This also raised the possibility that allopurinol may have some beneficial effects on the heart but the study was unable to prove that as it was not powered for change in blood markers. A possible explanation for the above finding is the improvement in cardiac energetics as mentioned in the previous section.

As expected, the serum uric acid level was reduced from baseline by allopurinol by 59% which is similar to previous studies[173,174] using similar dose of allopurinol.

4.3.4 Health related questionnaires

The St George Respiratory Questionnaire (SGRQ) is the most relevant quality of life questionnaire for this cohort of patients with chronic lung disease. There was no significant difference in the change in SGRQ total score between the allopurinol and the placebo group. This did not change when looking at SGRQ subdomains, i.e. symptom score, activity score and impact score. In addition, the change in SGRQ total score from baseline did not reach the minimal clinically important difference of 4 unit change in this study.

When looking at SF-36 which is a general quality of life measure questionnaire, there were also no significant differences in the change in all nine domains of the questionnaire between the allopurinol and the placebo group.

These results probably reflect the overall absence of beneficial effect of allopurinol in the studied population. Subgroup analyses like the ones done for the primary outcome did not change the results.

It is interesting that there was a statistically significant reduction in right ventricular mass in the subgroup of patients with more severe airflow limitation but no significant change in health related questionnaire scores in the same subgroup. This probably means that a change in ventricular mass may not translate into a change in quality of life. Obviously here, it is difficult to make this a definite conclusion. Further studies looking at this subgroup would help to clarify this.

In contrast, a statistically significant difference was observed in the TDI score between allopurinol (-0.7 ± 0.9) and placebo (-0.1 ± 0.9). This finding needs to be interpreted cautiously as only 54 of 63 completed cases (86%) were used for the analysis of TDI scores. It is difficult to explain the significant finding above when allopurinol did not have an overall impact on the primary outcome and the other secondary outcomes (including other quality of life questionnaire scores). The TDI score did not reach the 1-unit change from baseline in either group which is considered to be the minimal clinically important difference in clinical trials.[195] This statistically significant finding is not clinically significant and therefore, it would be wise to conclude that allopurinol is unlikely to be of clinical benefit in terms of improving breathlessness.

4.3.5 Spirometry

All the spirometric measurements and ratios (i.e FEV_1, FEV_1 %, FVC, FVC % and FEV_1/FVC, $FEF_{25\text{-}75}$ and $FEF_{25\text{-}75}$ %) were similar at baseline in both allopurinol and placebo groups. After 12 months of treatment, there was no significant difference in the change in the measurements between the two groups. There was also no significant change from the baseline values in both groups. These results are comparable to Ichinose et al.[182] study which demonstrated no change in FEV_1 after 10 patients with COPD was treated with 300 mg allopurinol for four weeks.

4.4 Strengths & Limitations

Both participants and investigators were blind to treatment. All cardiac MRI measurements were performed by a single investigator (JWM), eliminating inter-observer variability. There was similar number of withdrawals in both allopurinol and placebo groups (see CONSORT diagram, *Figure 21*). The total number of withdrawals was as predicted and the study was appropriately powered for the primary outcome measure.

One of the limitations of this study is that right heart catheterisation (RHC), which is the gold standard for assessing pulmonary haemodynaemics, was not done. RHC is invasive and it would introduce unnecessary risk and reduce the number of patients willing to be recruited, hence potentially making recruits atypical. Pulmonary acceleration time (PAT) is a good alternative and non-invasive echo measurement that has a correlation coefficient of r = 0.88 with pulmonary pressures.[216]

Another limitation is the lack of data on transfer factor of the lung for carbon monoxide. Transfer factor is a measure of the extent to which oxygen diffuses from the lungs into the blood stream. Reduction in transfer factor is very common in chronic lung disease due to the structural lung abnormalities. Pulmonary hypertension causes a further reduction in the transfer factor. If the transfer factor was measured, it would have helped to further characterise the study population. However, it was not an essential parameter as it was not expected that allopurinol would have any beneficial effect on transfer factor.

Post-hoc analyses of data are fraught with limitations. They conform to neither the population nor the randomisation model of statistical inference, which is the foundaton of randomised clinical trials.[217] The results from post-hoc analyses should be interpreted carefully as they might have been nothing more than simple coincidence.

4.5 Conclusion

In conclusion, there was no overall effect of high dose allopurinol on right ventricular hypertrophy in patients with pulmonary hypertension associated with chronic obstructive pulmonary disease or interstitial lung disease. There was also no overall effect of allopurinol on LV and RV measurements (by CMRI), on quality of life questionnaires, spirometry, six-minute walk test and blood markers.

There was a potential benefit of allopurinol in COPD patients with more severe airflow limitation (FEV_1 < 50% predicted). There was also a potential benefit of allopurinol in patients with higher NT-proBNP levels. Further studies are warranted to assess the longer-term impact of allopurinol in this population.

4.6 Future research

A study involving patients with a more strict criteria of at least severe airflow limitation would be the logical next step. The primary outcome would still be regression of right ventricular hypertrophy. This is because the aim of treating patients with pulmonary hypertension is to prevent progression of right ventricular hypertrophy to ventricular dilatation, and eventually to ventricular failure. The study would involve a treatment period of 24 months with repeating the outcome measures at half-way (that is, at 12 months). A longer duration of treatment may help to unmask whether ventricular regression may confer improvement in quality of life.

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6. Appendices

Appendix A - Letter of invitation with reply slip

[LOCAL HEADED PAPER]

Dear < Insert Patient name>,

Research Project: ALPHA Trial

Along with colleagues in the University of Dundee, the <Insert name of clinic and Hospital referral is from> is very active in clinical research and our team would like to let you know about a research project that you may be interested in. I am writing to you as you are being treated for COPD or interstitial lung disease.

Lung disease patients can sometimes develop heart complications in addition to their lung problems.

One of these complications is that the heart wall can thicken and the heart becomes less efficient. This can be difficult to detect and treat but is important because it can make people more breathless.

We are conducting a research study to see whether treating patients who have lung disease and who are found to have evidence of this heart thickening would benefit from taking a pill to reduce this thickening of the heart.

I enclose a brief Participant Information Sheet about the study and what it involves.

Please read the Participant Information Sheet carefully.

Please let me know if you are interested in participating in the study by returning the form attached in the enclosed stamped addressed envelope. We will pass your detail on to the researchers who will then contact you by phone to discuss the study and arrange a convenient time to meet you.

The study visits will be done at [LOCAL HOSPITAL].

With best wishes

Local Clinical Lead or PI Signature

Page 1 of 2 Clinic letter of invitation to participant: ALPHA Trial Version 3: 21st January 2016

[LOCAL HEADED PAPER]

Research Project: ALPHA Trial

Please only complete and return if you wish to participate in the study

Name…………………………………………………………………..…………

Address……………………………………..…………………………..…………..

……………………………………...…………..… Postcode ………...……......

Telephone number……………….…….

Best time to contact…………..…..

Appendix B - Patient information sheet (PIS)

PARTICIPANT INFORMATION SHEET

Title of Study

Does allopurinol reduce right ventricular mass in lung disease associated pulmonary hypertension?
The ALPHA Trial.

Name of Researchers

Chief Investigator: Professor Allan Struthers

Principal Investigator: Dr Patrick Liu

Details of Study

You are being invited to participate in a clinical trial being sponsored by the University of Dundee and NHS Tayside. This study will form part of a doctorate in medicine (MD) degree for Dr Liu. Before you decide whether or not to take part it is important for you to understand why the research is being carried out and what it involved. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is unclear or if you would like more information. Take your time to decide whether or not you would like to take part.

Background

People with lung disease are at increased risk of heart complications. One of the biggest problems they can have is that the muscle wall of the heart thickens.

The medical term for this is Right Ventricular Hypertrophy (RVH). RVH makes the heart less efficient and patients with RVH are at a greater risk of heart complications than those without it. The pressure in the blood vessel between this heart chamber and the lungs can also increase, called pulmonary artery hypertension (PAH). This can leave patients more breathless as the blood is not picking up oxygen well from the lungs.

It has previously been shown that a drug called allopurinol, which is usually used to treat gout, had the remarkable side effect of being able to reduce thickening of the left side of the heart wall in patients who had kidney disease or diabetes. The aim now is to see if patients with lung disease and raised pressure in their right side of the heart may also benefit from treatment with allopurinol. If RVH can be reduced using allopurinol, this might be a new way to reduce the risk of cardiac events such as heart attack or stroke in these patients and might possibly improve their exercise ability and symptoms.

In this study the aim is to recruit patients who have COPD or interstitial lung disease and who may have raised PAH. All will be screened for PAH by doing an ultrasound scan of the heart and then that will be confirmed with a Magnetic Resonance Imaging (MRI) scan, which is a special scan of the heart using an MRI machine to measure the extent of thickening of the heart muscle. All the patients currently prescribed medication for their lung disease will continue as normal on that. They will have a further MRI scan when their treatment with allopurinol or placebo finishes after 9 to 10 months. We are aiming to recruit 72 patients in Tayside and Grampian.

Do I have to take part?

Participation in this study is entirely voluntary and you are free to refuse to take part or withdraw from the study at any time (without having to give a reason) and without this in any way affecting your future medical care or your relationship with medical staff looking after you. Some insurance companies consider that participation in medical research such as this is a "material fact" which should be mentioned in any proposal for health-related insurance or which could influence their judgement in consideration of claims under existing policies. You should check that participation in this research does not affect any policy you might be thinking about taking out or any existing policy.

What is involved in the study?

This study takes between 9 to 10 months for you to complete with visits for the study scheduled to take place at your convenience. It will involve 7 appointments lasting approximately 30 mins to 2 hours. A maximum of 3 visits will be in the hospital whilst the others can be arranged at your home if this is more convenient for you.

This study is a randomised, double blinded, placebo controlled study to be conducted at Ninewells Hospital & Medical School, Dundee. You will be given a tablet which contains the medication we are testing (called allopurinol) or an inactive tablet (called a placebo).

Before you start on any study medication the doctor will do a screening visit to check you are eligible for the study. A summary of the tests done is included in a diagram at the end of this information sheet.

Visit 1 Screening

The screening period may take between 1 and 4 weeks whilst the doctor assesses your suitability for the trial. Typically this may involve one or two visits.

At the first screening visit the doctor will check your medical history, do a clinical examination and do an ultrasound scan of your heart to check if you have evidence of RVH and/or PAH. This is a painless procedure where the doctor will put some gel on your chest wall and use an ultrasound scanner to get a picture of your heart. If you do not have RVH or PAH you will be unable to take part further in this study further.

Should you have RVH and/or PAH the doctor or research nurse will check your blood pressure and pulse readings and do some routine blood tests to assess your liver and kidney function and urate (the molecule that causes gout) to be sure that it is safe for you to participate.

The total volume of blood taken at this visit will be about 17ml (3 teaspoons full).

If able, you will be asked to walk for six minutes at your own pace and the distance you walk in that time will be measured (six minute walk test).

If you are eligible on the ultrasound scan and all your blood tests tell the doctor it is safe for you to proceed you will be asked to return for an MRI Scan- a special scan of your heart (further details below).

Baseline MRI Scan

For the MRI Scan you will receive an appointment either by telephone call, letter or e-mail and be sent directions to attend the MRI department of the Clinical Research Centre, Ninewells Hospital, Dundee. If you are a woman of child bearing potential you will be asked to provide a urine specimen which you can either bring with you (a specimen bottle will be provided) or you could provide a sample at the beginning of your MRI clinic visit. A pregnancy test will be performed to ensure your safety. A positive pregnancy test will exclude you from having an MRI scan and you will be unable to take further part in this study.

Before your scan you will meet one of the research team who will check that you are eligible to have the scan and who will obtain your written consent. You will then be seen by the radiographer, the person taking your scan, and she/he will help you to complete a checklist about matters that might prevent you from having the scan.

If you are found to have a history of a penetrative eye injury or exposure to metal fragments in your eye(s) you will be asked to consider having an eye x-ray prior to your MRI scan to establish safety. This can be performed at the main x-ray department at the same visit prior to your MRI scan. You will be taken or directed to the x-ray department. If the radiology staff establishes that you are unsafe to scan you will not have your MRI and you will be free to go. With your consent, we will write to your GP informing them of your MRI safety status, as this information may be of benefit for your future health care needs.

If you are found to have NO history of a penetrative eye injury and have had NO exposure to metal fragments in your eye(s), or otherwise, the radiology staff will establish that it is SAFE to scan you. You will proceed to have your MRI whereupon you will then be asked to change into a gown for the scan. After being prepared for your scan, you will be asked to lie up on the scanning table and then will be moved into the centre of the scanner (the scanner is shaped like a big doughnut). During the scan, which takes around 45 minutes, you will be able to speak to the radiographer. The scan will take pictures of your heart and blood vessels. As the scan is noisy you will be wearing hearing protection. After you have completed the scan you are free to go home. You can drive if you need to or if you prefer a taxi can be arranged to take you to and from the hospital. A specialist will examine your scan at a later date for any signs of disease and will measure your right ventricular mass.
This MRI visit should take no longer than one and a half hours.

Visit 2 Baseline

This visit will take place after the MRI Scan has been done anytime up to two weeks after the MRI scan but usually on the day of it. It will include the following investigations:

Vital signs: checks of your blood pressure, pulse, height and weight.

Blood tests: There are two types of blood tests in this trial, one to ensure your safety before and during treatment with the study medication, as noted at visit 1 and follow up visits, and the other taken for research purposes. These research bloods (20ml; about 4 teaspoons) will be used to test for urate levels and also tell us how well your heart may be responding to treatment. These are tests that are not done routinely in NHS labs but may be of use in helping us decide which blood tests should be used in this condition in future. One 20ml sample will be taken at this baseline visit and the other when you complete the trial. Following completion of the trial we may test for additional tests of interest on any left-over research blood which will be stored locally and at the end of the study transferred and will be stored anonymously in the secure University of Dundee laboratory in the Division of Cardiovascular and Diabetes Medicine.

Genetic analysis blood test: We will ask you to provide one 10ml blood sample for storage. The sample will be fully anonymised and will be subject to approval of a Research Ethics Committee prior to future analysis. The results of any future genetic tests would not be linked to your records and you would not receive any information about the results. You can opt not to have this done without affecting your participation in the study. This sample will be stored locally and at the end of the study transferred and stored in the secure laboratory within the Division of Cardiovascular and Diabetes Medicine at Ninewells Hospital.

Lung Function Tests: We will do some simple tests to measure your lung capacity and how well your lungs are working.

Six Minute Walk Test: If able, you will be asked to walk for six minutes at your own pace and the distance you walk in that time will be measured. If you need to stop during the test for a rest, that is fine or if you are unable to walk for the full six minutes a note will be taken of how long you managed and how far you walked.

Quality of Life questionnaires: During the visit you will be asked to complete three or four short questionnaires which may allow an assessment of how your lung disease impacts on your quality of life.

At the end of this first drug dosing visit you will be randomly assigned to either low dose allopurinol (100mg) or placebo. The tablets allocated to you are decided in a random way (a bit like tossing a coin) such that neither you nor the research staff will know which tablet you are taking at any time until after the study is completed. This ensures that the study results cannot be influenced by knowing whether you are receiving the medication or not.

You will be given enough study drug to take once daily for one month.

Visit 3

Visit 3 will occur about 2 weeks after visit 2. At this visit you will have blood tested for safety measures, have your vital signs measured and the doctor will assess if you have had any problems on the study medications. At the end of the visit you will receive a higher dose of allopurinol or placebo (300mg once daily) for four weeks.

Visit 4

Visit 4 will occur at about four weeks from visit 3. At this visit safety blood tests will be taken and your vital signs will be checked by the doctor to ensure there are no adverse events whilst on the study medication. At the end of the visit you will receive a higher dose of allopurinol or placebo (300mg twice daily) for the duration of the study and will get two months supply to take home with you.

Visits 6 & 8

Visits 6 and 8 will occur at three months and six months from the baseline randomisation visit. At both these visits safety blood tests will be taken and your vital signs will be checked by the doctor to ensure there are no adverse events whilst on the study medication. At the end of the visit you will receive enough supplies of medication to take you to your next study visit.

Visits 5 and 7 (Telephone Calls)

In order to reduce the planned number of visits for you to the hospital during this trial, there are two telephone visits scheduled at week 8 and month 6 after baseline. On these dates the study doctor or one of the research team will phone you to check how you are doing on the study medication. If there are any concerns at all, they will invite you up to Ninewells Hospital to be checked over by the doctor.

Final Visit 9

This is the final study visit. At this visit the final urate and safety blood tests will be done, you will do the six minute walk test, will have an MRI scan (within 2 weeks of the visit) and have your PFTs measured again. These will ultimately be compared with the baseline measurements taken before you started on study medication. We will also take a 20ml sample of blood for research purposes as described at the baseline visit.

For all visits noted above the doctor will assess you for any side effects of the medication and will check your vital signs and do blood tests to assess if the allopurinol has caused any problems. Note that if you have tolerated the lower 300mg daily dose of medication and then develop problems tolerating the higher 300mg twice daily dose the doctor will discuss you returning to the lower dose with your agreement.

Home Visits

The study visits 3, 4, 6 and 8 can be arranged at your home for your convenience. For these visits, the doctor or research nurse will check your vital signs and take safety blood tests to ensure there are no adverse events whilst on the study medication. At the end of the visit, you will receive enough supplies of the medication to take you to your next study visit.

Medication being tested

The medication used in this study is called allopurinol. It was been around for about 50 years now although mainly for the treatment of gout. It has a good safety record and is generally well tolerated. However, like most medicines, allopurinol occasionally causes side effects. The most common side effect is nausea and some abdominal discomfort which affects less than one in ten of patients on allopurinol. This can be minimised by taking the tablets with food.

Allopurinol causes a skin rash in one in a hundred or less of patients. This may be associated with fever, swollen glands, joint pains, unusual blistering or bleeding. Were any of these symptoms to develop, you should stop taking the tablet immediately and contact the study doctor as soon as possible. You may also seek advice from your GP.

Reports of other side effects of allopurinol are very rare (less than 1 in 10,000 people) and it is not always clear if they are truly related to the treatment. The complete range of reported side effects is set out in a Patient Information Leaflet, a copy of which will be given to you at your screening visit for your information, but include headache, stomach upset, drowsiness, anaemia. This will be further discussed with you before you make a final decision about taking part in this study.

Contraceptive Advice

Anyone who is pregnant cannot take part in this study. If you are a woman of childbearing age we will need to do a pregnancy test before the study. It is also important that you do not become pregnant during the study. We will do a urine pregnancy test at all clinic visits if you are a women of childbearing potential and are sexually active. Here is some advice on contraception. To avoid getting pregnant, not having sex at all is obviously effective. If you follow this strictly, no contraception is needed. If not, these are effective types of contraception:

- Combined Oral Contraceptive Pill
- EVRA-osetrogen and progestogen: 'Transdermal Patch'
- Progestogen only pill: 'mini pill'
- Depoprovera injection (medroxyprogesterone acetate)
- Implanon Implant (Etonogestrel)
- Mirena Coil (Intra-Uterine System)
- IUD-copper containing intrauterine device
- Female sterilisation

Male vasectomy is also a good form of contraception but only if the procedure has been checked afterwards by your doctor to make sure it has worked. No contraception method is 100% reliable by itself. Even surgical sterilisation in men and women has been known to fail very occasionally. We advise using additional contraception from the start of the study.

You may normally use 'barrier methods' such as the condom, diaphragm or cap. There is no definite proof that using a spermicide with a 'barrier method' gives extra protection but some condoms are manufactured with spermicide on them. If you require further advice on contraception, please ask.

What are the discomforts, risks and side effects?

The side effects of the allopurinol are discussed under the 'medication' section above.

Having blood tests taken can cause some mild bruising and temporary discomfort.

MRI scanning: This type of scan is very safe and does not use radiation. Some people, when being scanned, may feel a bit closed in but you will be in constant contact with the person performing the scan and you can come out at any time. The scanner is a bit noisy but you will be given ear protection which also plays music.

What are the benefits of taking part in the study?

You will be monitored closely during the study and will be seen by a doctor at each of your study visits. Besides having tests that have already been mentioned, your medication will be reviewed on a regular basis. The tests will give us information about the function of your heart, kidneys and blood circulation. If any of these investigations, including information from the MRI scan of your heart reveal any new abnormality we will either discuss this with your hospital consultant or refer you to a specialist clinic (whichever seems most appropriate). The study will not immediately benefit you, but if the results of the study are positive it may change the practice of managing patients with treated lung disease who may have RVH, like you and potentially will have a great impact on other such patients in the future. If so, you may gain eventually from our discovering a new treatment for your condition.

Complaints, Insurance and indemnity

Right to raise concerns

If you have any concerns about your participation in the study you have the right to raise your concern with a researcher involved in conducting the study or a doctor involved in your care.

Right to make a complaint

If you have a complaint about your participation in the study, you should first talk to a researcher involved in the study. However you have the right to raise a formal complaint. You can make a complaint to a senior member of the research team or to the Complaints Officer for NHS Tayside.

Complaints and Claims Manager
Complaints and Advice Team
Level 7, Ninewells Hospital
Dundee DD1 9SY
Freephone: 0800 027 5507
Email: nhstaysidecomplaints@thb.scot.nhs.uk

Right to make a claim

In the event that you think you have suffered harm as a result of your participation in the study there are no automatic financial compensation arrangements. However, you may have the right to make a claim for compensation against the University of Dundee or NHS Tayside. Where you wish to make a claim, you should consider seeking independent legal advice but you may have to pay for your legal costs.

Insurance

The University of Dundee maintains a policy of professional negligence clinical trials insurance which provides both legal liability cover and no fault compensation in respect of accidental injury. Tayside Health Board is a member of the Clinical Negligence and Other Risks Insurance Scheme which provides legal liability cover.

You may be required to inform insurance companies with whom you intend to purchase life insurance, income protection or travel insurance, about your participation in this study. It is not anticipated that your involvement in the study will adversely affect your ability to purchase insurance but some insurers may use this information to limit the offer of cover, apply exclusions or increase any premium.

Will the research influence the treatment I receive?

The research will not immediately alter the regular treatments you currently receive.

Will my taking part in the study be kept confidential?

Your personal data will be kept confidential. With your permission, identifiable information about you and data collected during the study will be held securely by the University of Dundee / University of Aberdeen and under the control of the local Principal Investigator. All data collected in this study will be coded and stored on a computer system protected by a password only available to the researchers. No one outside the research team will have access to any identifiable information and all identifiable information and data will be kept securely. Your data will be archived securely for at least five years after the end of study as this is a legal requirement for drug studies. With your permission, we will inform your GP of your participation in this study. It is a requirement of the regulatory authority for clinical trials that your records in this study, together with any other relevant medical records, be made available for scrutiny by appropriate staff from NHS Tayside, University of Dundee (or their appointed third party) and the regulatory authority themselves.

Additionally there will be two sets of information obtained after you have had your MRI scan. One set will be the MRI scan images and the other, the research data obtained from those images. The MRI images obtained will be stored indefinitely using your name and unique hospital record number within the NHS clinical system and can be made available to specialist doctors for your future health care needs. Your research data will be stored using a unique study code which is non-identifiable and held on password protected University of Dundee / University of Aberdeen secure databases. Only individuals directly involved with the study will have access to this information.

Will I continue to receive the medication used in this study after it finishes?

No. The study is designed to give an indication of possible benefit from the medicine being tested and it may be some time before we can be sure about how useful it actually is.

Expenses

Taxi transport, or reasonable costs to cover your travel costs, will be provided for any extra visits to the hospital for the purposes of this study.

Who has reviewed this study?

The East of Scotland Research Ethics Committee, REC 2, which has the authority to scrutinise proposals for medical research on humans, has examined this study and has raised no objections from the point of view of medical research.

It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from The University of Dundee, NHS Tayside and by the Regulatory Authorities, whose role it is to check that research is properly conducted and the interests of those taking part are adequately protected.

Who has funded this study?

The British Heart Foundation has funded this study.

Contact details for the study Doctor.

If you are worried at any time about the research or wish to discuss things generally further, please do not hesitate to contact:

Dr Patrick Liu
Clinical Research Fellow
University of Dundee
Division of Cardiovascular and Diabetes Medicine
Medical Research Institute
Mail Box 2, Ninewells Hospital and Medical School
Dundee DD1 9SY

Tel: (01382) 383473

Email: p.s.k.liushiucheong@dundee.ac.uk

Contact Numbers if unwell during the trial

If during the study you become unwell or are concerned, you can contact the study team during normal working hours on (01382) 383473 If you are unwell and need urgent advice or assistance do not delay in seeking further advice or treatment as usual through the NHS services such as NHS24 (111) or by contacting your GP who will have received details of your participation in this study should you agree to them being informed.

Thank you for reading this information sheet and considering taking part in this study. If you would like more information or want to ask questions about the study please contact the study team on the number above.

What will happen to me during the study?

The following diagram is the programme of visits involved in this study.

VISIT	Visit 1 Screening	Visit 2 Baseline/ Randomisation	Visit 3	Visit 4	Visit 5 Tele Call	Visit 6	Visit 7 Tele Call	Visit 8	Visit 9 Final Visit
	-1 to -4 weeks	Day 0	2 week (+/- 3 days)	6 week (+/- 1 week)	8 week (+/- 3 days)	Month 3 (+/- 1 week)	Month 6 (+/- 2 weeks)	Month 9 (+/- 2 weeks)	Month 9-10
Daily drug dose if increasing		100mg/ placebo	300mg/ placebo	600mg/ placebo		600mg/ placebo		600mg/ placebo	
Informed Consent	X								
Doctor to check your Medical & Family History	X								
Doctor to do Clinical Examination	X								
Vital Signs- Blood pressure, pulse, height, weight	X	X	X	X		X		X	X
Echo cardiography	X								X
MRI Scan		X							X
Safety Blood Tests & Urate test (17ml)	X		X	X		X		X	X
Research Bloods, BNP & Urate Test (20ml)		X							X
Genetic blood sample (if consented) (10ml)		X							
Urine Pregnancy Test (if required)	X	X	X	X		X		X	X
Lung function Tests		X							X
6 Min Walk Tests	X	X							X
Questionnaires		X							X
Get new supply of study medication		X	X	X		X		X	
Doctor to check any problems since last visit		X	X	X	X	X	X	X	X

Appendix C - Participant information leaflet (PIL)

WHO IS ORGANISING AND FUNDING THIS RESEARCH?

The study has been organised by Professor Allan Struthers and Dr Patrick Liu who are based in Ninewells Hospital & Medical School in Dundee.

The study is funded by the British Heart Foundation.

All information collected about you during the study will be kept strictly confidential in accordance with the Data Protection Act 1998.

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NHS Tayside

Brief Participant Information Sheet v3.0, 06/08/2015

FURTHER INFORMATION:

Call Researcher:
Dr Patrick Liu on 01382 383473
Email:
p.s.k.liushiucheong@dundee.ac.uk

Alternatively you can call:
Professor Allan Struthers on 01382 383013

Thank you very much for taking the time to read this leaflet.

This study has been reviewed by the East of Scotland Research Ethics Committee REC 2 who have raised no objection from the point of view of medical ethics.

DO YOU HAVE LUNG DISEASE?

BEEN ON STABLE TREATMENT FOR MORE THAN 2 WEEKS?

THIS RESEARCH STUDY MAY BE OF INTEREST TO YOU ....

ALPHA Trial

Lung Disease Research Study

WHY ARE WE DOING THIS STUDY?

Patients with lung disease may develop thickening of the heart wall. This thickening of the heart wall can cause an increased risk of heart attack or stroke. It can though sometimes be reversed with appropriate medical treatment. This study will examine lung disease patients to see if they have this heart wall thickening and if they do will invite them into a drug trial to test if one years treatment with allopurinol can improve it.

WHO CAN TAKE PART?

- Aged 18 or over
- Being treated for COPD or interstitial lung disease
- Been on lung disease medications for at least two weeks that have not changed

WHAT IS BEING TESTED?

Allopurinol

Allopurinol is a medicine usually given for treating gout. It has been used by doctors for over 50 years.

Other research suggests that allopurinol might be able to improve thickening of the heart wall.

WHAT WILL HAPPEN IF I TAKE PART?

We will do an ultrasound scan of your heart to check if the heart wall is thickened and will ask about your general health and the medicines you take to confirm the study is suitable for you.

If you are suitable to take part an MRI scan of your heart will be arranged to get a more accurate measurement of the extent of this heart wall thickening. After a year of treatment this will be repeated to see if it has improved.

You will be in the study therefore for around one year. It will involve seven appointments lasting approximately 30 mins to 2 hours. A maximum of 3 visits will be in the hospital whilst the others can be arranged at your home if this is more convenient for you. A taxi can be arranged to take you to all of the hospital visits or we will reimburse your travel expenses.

You will be asked to take one capsule of the study medicine twice daily. This will contain either allopurinol or a placebo (dummy) medicine. This will be chosen at random.

You can take all your usual medicines during the study.

WHAT ARE THE RISKS AND BENEFITS OF TAKING PART?

Allopurinol can sometimes cause a rash. If this does occur your study medications will be stopped.

You will be closely monitored for side effects and have regular blood tests to ensure your kidneys and liver are functioning well.

The study may not immediately benefit you but the results may lead to further research aimed at improving the care of lung disease patients.

Some people may not be able to take part due to specific medical conditions or medications. To find out more please call the researchers.

British Heart Foundation

Appendix D - Consent form

Study Number: (EudraCT) 2014 -002305-38
Participant Identification Number for this trial:

PARTICIPANT CONSENT FORM

Title of Study: **Does Allopurinol reduce Right Ventricular Mass in Lung Disease associated Pulmonary Hypertension? (The ALPHA Trial)**

Name of Researchers:

Chief Investigator: Professor Allan Struthers
Principal Investigator: Dr Patrick Liu Shiu Cheong

Please initial box

1. I confirm that I have read and understand the information sheet dated___________ (version ___) for the above study. I have had the opportunity to consider the information, to ask questions, and have had them answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by the research team or from the regulatory authorities, NHS Tayside, or the University of Dundee (or their appointed third party), where it is relevant to my taking part in this study. I give permission for these individuals to have access to my records.

4. I agree to my GP being informed of my participation in this study. YES/NO (*please circle*)

5. I understand and agree that the data that I provide will be gifted by myself, and analysed by members of the study team or stored (anonymised) for up to 15 years and can be used for future, as yet unspecified, medical research into health, illness and medical treatment.

6. *OPTIONAL*
   I agree to donate an additional 10ml of blood for genetic research purposes and understand that this may be stored indefinitely for future research use.
   YES/NO (*please circle*)

7. *OPTIONAL*
   I agree any spare blood left over from the 20ml blood sample taken for research purposes at the start and end of the study may be stored indefinitely for future research use. YES/NO (*please circle*) ☐

8. I agree to take part in the above study. ☐

Name of participant	Date	Signature
Name of person taking consent	Date	Signature

Original to be kept with TMF, 1 copy for participant; 1 copy for hospital notes

Appendix E - Letter to GP

Date: *<insert date>*

Dear *<Insert GPs details>*

Title of Project: Does Allopurinol reduce Right Ventricular Mass in Lung Disease associated Pulmonary Hypertension. **(The ALPHA Trial)**

Name of Investigator: Dr Patrick Liu Shiu Cheong

Patient details: *<Insert patient details including CHI number and address>*

The patient named above has agreed to take part in this clinical research study.

This double blind randomised placebo controlled trial will assess the effect of allopurinol on Right Ventricular Hypertrophy as measured by cardiac MRI in patients with COPD or Interstitial Lung Disease.

Their participation in this study will last for between 12 to 13 months.

I refer you also to some medications that require caution before prescribing whilst your patient is on this trial.

6-mercaptopurine or azathioprine: concurrent prescription of either of these drugs is not allowed due to the known interaction of these drugs with allopurinol. If your patient needs to start on treatment with either of these whilst receiving study medication then they would be withdrawn from the study.

Theophylline: due to the possible influence of allopurinol on theophylline levels any participants already on this drug should be excluded at screening. If they need to start on treatment with theophylline whilst receiving study medication then they would be withdrawn from the study.

Ampicillin/amoxicillin: are not prohibited, but an increase in frequency of skin rash has been reported among patients receiving ampicillin or amoxicillin concurrently with allopurinol compared to patients who are not receiving both drugs. The cause of the reported association has not been established. However, in participants receiving allopurinol an alternative to ampicillin or amoxicillin should be used where available.

I have enclosed a patient information sheet which gives full study details, however if there are any questions you may have regarding the study, I would be happy for you to contact me.

Yours faithfully

Dr Patrick Liu Shiu Cheong
BHF Clinical Research Fellow
Tel: 01382 383473

Appendix F - St George's Respiratory Questionnaire (SGRQ)

ALPHA Trial

Participant ID : | | |

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE
ORIGINAL ENGLISH VERSION

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE (SGRQ)

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about your answers.

Date Completed: D D / M M / Y Y Y Y

Before completing the rest of the questionnaire:

Please tick in one box to show how you describe your current health:

Very good	Good	Fair	Poor	Very poor
☐	☐	☐	☐	☐

UK/ English (original) version

continued…

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P.W. Jones, PhD FRCP
Professor of Respiratory Medicine,
St. George's University of London,
Jenner Wing,
Cranmer Terrace,
London SW17 ORE, UK.

Tel. +44 (0) 20 8725 5371
Fax +44 (0) 20 8725 5955

ALPHA Trial

Participant ID :

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest trouble you have had over the past 3 months.

Please tick (✓) *one box for each question:*

		most days a week	several days a week	a few days a month	only with chest infections	not at all
1.	Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2.	Over the past 3 months, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3.	Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4.	Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✓) *one:*

more than 3 attacks ☐
3 attacks ☐
2 attacks ☐
1 attack ☐
no attacks ☐

6. How long did the worst attack of chest trouble last?
(Go to question 7 if you had no severe attacks)

Please tick (✓) *one:*

a week or more ☐
3 or more days ☐
1 or 2 days ☐
less than a day ☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) *one:*

No good days ☐
1 or 2 good days ☐
3 or 4 good days ☐
nearly every day is good ☐
every day is good ☐

8. If you have a wheeze, is it worse in the morning?

Please tick (✓) *one:*

No ☐
Yes ☐

ALPHA Trial

Participant ID :

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest trouble you have had over the past 3 months.

Please tick (✓) *one box for each question:*

	most days a week	several days a week	a few days a month	only with chest infections	not at all
1. Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2. Over the past 3 months, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3. Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4. Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✓) *one:*

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest trouble last?
(Go to question 7 if you had no severe attacks)

Please tick (✓) *one:*

- a week or more ☐
- 3 or more days ☐
- 1 or 2 days ☐
- less than a day ☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) *one:*

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- nearly every day is good ☐
- every day is good ☐

8. If you have a wheeze, is it worse in the morning?

Please tick (✓) *one:*

- No ☐
- Yes ☐

ALPHA Trial

Participant ID : ☐☐☐

St. George's Respiratory Questionnaire
PART 2

Section 1

How would you describe your chest condition?

Please tick (✓) *one*:

- The most important problem I have ☐
- Causes me quite a lot of problems ☐
- Causes me a few problems ☐
- Causes no problem ☐

If you have ever had paid employment.

Please tick (✓) *one*:

- My chest trouble made me stop work altogether ☐
- My chest trouble interferes with my work or made me change my work ☐
- My chest trouble does not affect my work ☐

Section 2

Questions about what activities usually make you feel breathless <u>these days</u>.

Please tick (✓) in *each box* that applies to you *these days*:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

ALPHA Trial

Participant ID :

St. George's Respiratory Questionnaire
PART 2

Section 3

Some more questions about your cough and breathlessness these days.

Please tick (✓) in *each box* that applies to you *these days*:

	True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest trouble may have on you these days.

Please tick (✓) in *each box* that applies to you *these days*:

	True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication, if you are receiving no medication go straight to section 6.

Please tick (✓) in *each box* that applies to you *these days*:

	True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

ALPHA Trial

Participant ID :

St. George's Respiratory Questionnaire
PART 2

Section 6

These are questions about how your activities might be affected by your breathing.

Please tick (✓) in *each box* that applies to you *because of your breathing*:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐

Section 7

We would like to know how your chest usually affects your daily life.

Please tick (✓) in *each box* that applies to you *because of your chest trouble*:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

ALPHA Trial

Participant ID :

Date Completed: D D / M M / Y Y Y Y

St. George's Respiratory Questionnaire

Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church, pub, club or place of entertainment

Going out in bad weather or into smoky rooms

Visiting family or friends or playing with children

Please write in any other important activities that your chest trouble may stop you doing:

....

....

....

....

Now would you tick in the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐

It stops me doing one or two things I would like to do ☐

It stops me doing most of the things I would like to do ☐

It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish would you please check to see that you have answered all the questions.

Participant Initial's: ____________________ Date : ________________

Interviewers Name: ____________________ Signature : ______________ Date : ________________

Appendix G - Short Form 36 (SF-36) Health survey

ALPHA Trial

Participant ID :

RAND 36-ITEM HEALTH SURVEY

Date Completed: D D / M M / Y Y Y Y

1. In general, would you say your health is:	
Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

2. **Compared to one year ago,** how would your rate your health in general **now**?	
Much better now than one year ago	1
Somewhat better now than one year ago	2
About the same	3
Somewhat worse now than one year ago	4
Much worse now than one year ago	5

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

(Circle One Number on Each Line)

	Yes, Limited a Lot	Yes, Limited a Little	No, Not limited at All
3. **Vigorous activities,** such as running, lifting heavy objects, participating in strenuous sports	[1]	[2]	[3]
4. **Moderate activities,** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	[1]	[2]	[3]
5. Lifting or carrying groceries	[1]	[2]	[3]
6. Climbing **several** flights of stairs	[1]	[2]	[3]
7. Climbing **one** flight of stairs	[1]	[2]	[3]
8. Bending, kneeling, or stooping	[1]	[2]	[3]
9. Walking **more than a mile**	[1]	[2]	[3]
10. Walking **several blocks**	[1]	[2]	[3]
11. Walking **one block**	[1]	[2]	[3]
12. Bathing or dressing myself	[1]	[2]	[3]

ALPHA Trial

Participant ID :

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

(Circle One Number on Each Line)

	Yes	No
13. Cut down the amount of time you spent on work or other activities	1	2
14. **Accomplished less** than you would like	1	2
15. Were limited in the **kind** of work or other activities	1	2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

(Circle One Number on Each Line)

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	1	2
18. **Accomplished less** than you would like	1	2
19. Didn't do work or other activities as **carefully** as usual	1	2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

(Circle One Number)

Not at all 1

Slightly 2

Moderately 3

Quite a bit 4

Extremely 5

ALPHA Trial

Participant ID :

21. How much **bodily** pain have you had during the **past 4 weeks**?

(Circle One Number)

None 1

Very mild 2

Mild 3

Moderate 4

Severe 5

Very severe 6

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

(Circle One Number)

Not at all 1

A little bit 2

Moderately 3

Quite a bit 4

Extremely 5

ALPHA Trial

Participant ID :

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks . . .

(Circle One Number on Each Line)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
23. Did you feel full of pep?	1	2	3	4	5	6
24. Have you been a very nervous person?	1	2	3	4	5	6
25. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
26. Have you felt calm and peaceful?	1	2	3	4	5	6
27. Did you have a lot of energy?	1	2	3	4	5	6
28. Have you felt downhearted and blue?	1	2	3	4	5	6
29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?	1	2	3	4	5	6

32. During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

(Circle One Number)

All of the time 1

Most of the time 2

Some of the time 3

A little of the time 4

None of the time 5

ALPHA Trial

Participant ID :

How **TRUE** or **FALSE** is each of the following statements for you.

(Circle One Number on Each Line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
33. I seem to get sick a little easier than other people	1	2	3	4	5
34. I am as healthy as anybody I know	1	2	3	4	5
35. I expect my health to get worse	1	2	3	4	5
36. My health is excellent	1	2	3	4	5

Participant Initial's : ____________________ Date : ________________

Interviewers Name : ____________________ Signature : ______________ Date : ________________

Appendix H - King's Brief ILD Questionnaire (K-BILD)

ALPHA Trial

Participant ID :

King's Brief ILD Questionnaire (K-BILD)

This questionnaire is designed to assess the impact of your lung disease on various aspects of your everyday life. Read each question carefully and answer by SELECTING the response that best applies to you. Please answer ALL questions, as honestly as you can.

Date: ....

ALPHA Trial K-BILD Version 1.0 2nd March 2015

ALPHA Trial

Participant ID :

1. In the last 2 weeks, I have been breathless climbing stairs or walking up an incline or hill.

1. Every time
2. Most times
3. Several Times
4. Sometimes
5. Occasionally
6. Rarely
7. Never

2. In the last 2 weeks, because of my lung condition, my chest has felt tight.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

3. In the last 2 weeks have you worried about the seriousness of your lung complaint?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

4. In the last 2 weeks have you avoided doing things that make you breathless?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

5. In the last 2 weeks have you felt in control of your lung condition?

1. None of the time
2. Hardly any of the time
3. A little of the time
4. Some of the time
5. A good bit of the time
6. Most of the time
7. All of the time

6. In the last 2 weeks, has your lung complaint made you feel fed up or down in the dumps?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

7. In the last 2 weeks, I have felt the urge to breathe, also known as 'air hunger'.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

8. In the last 2 weeks, my lung condition has made me feel anxious.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

ALPHA Trial

Participant ID :

5. A little of the time
6. Hardly any of the time
7. None of the time

9. In the last 2 weeks, how often have you experienced 'wheeze' or whistling sounds from your chest?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

10. In the last two weeks how much of the time have you felt your lung disease is getting worse?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

11. In the last 2 weeks has your lung condition interfered with your job or other daily tasks?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

12. In the last 2 weeks have you expected your lung complaint to get worse?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time

13. In the last 2 weeks, how much has your lung condition limited you carrying things, for example, groceries?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

14. In the last 2 weeks, has your lung condition made you think more about the end of your life?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

15. Are you financially worse off because of your lung condition?

1. A significant amount
2. A large amount
3. A considerable amount
4. A reasonable amount
5. A small amount
6. Hardly at all
7. Not at all

Thank you for completing this questionnaire

Appendix I - Baseline/Transition Index (BDI-TDI)

ALPHA Trial

Participant ID :

Baseline/Transition Dyspnea Index (BDI/TDI)

BASELINE DYSPNEA INDEX

Baseline Functional Impairment

Date Completed: D D M M Y Y Y Y

____Grade 4	*No Impairment*	Able to carry out usual activities and occupation without shortness of breath.
____Grade 3	*Slight Impairment*	Distinct impairment in at least one activity but no activities completely abandoned. Reduction, in activity at work *or* in usual activities, that seems slight or not clearly caused by shortness of breath.
____Grade 2	*Moderate Impairment*	Subject has changed jobs *and/or* has abandoned at least one usual activity due to shortness of breath.
____Grade 1	*Severe Impairment*	Subject unable to work or has given up most or all usual activities due to shortness of breath.
____Grade 0	*Very Severe Impairment*	Unable to work *and* has given up most or all usual activities due to shortness of breath.
____W	*Amount Uncertain*	Subject is impaired due to shortness of breath, but amount cannot be specified. Details are not sufficient to allow impairment to be categorised.
____X	*Unknown*	Information unavailable regarding impairment.
____Y	*Impaired for Reasons Other than Shortness of Breath*	For example, musculoskeletal problem or chest pain.

Usual activities refer to requirements of daily living, maintenance or upkeep of residence, yard work, gardening, shopping, etc.

ALPHA Trial BDI-TDI Version 1 12th March 2015

BDI-TDI - United States/English
BDI-TDI_AU1.0_eng-USori.doc

ALPHA Trial

Participant ID : [ ] [ ] [ ]

Baseline Magnitude of Task

____Grade 4	*Extraordinary*	Becomes short of breath only with extraordinary activity such as carrying very heavy loads on the level, lighter loads uphill, or running. No shortness of breath with ordinary tasks.
____Grade 3	*Major*	Becomes short of breath only with such major activities as walking up a steep hill, climbing more than three flights of stairs, or carrying a moderate load on the level.
____Grade 2	*Moderate*	Becomes short of breath with moderate or average tasks such as walking up a gradual hill, climbing fewer than three flights of stairs, or carrying a light load on the level.
____Grade 1	*Light*	Becomes short of breath with light activities such as walking on the level, washing, or standing.
____Grade 0	*No Task*	Becomes short of breath at rest, while sitting, or lying down.
____W	*Amount Uncertain*	Subject's ability to perform tasks is impaired due to shortness of breath, but amount cannot be specified. Details are not sufficient to allow impairment to be categorised.
____X	*Unknown*	Information unavailable regarding limitation of magnitude of task.
____Y	*Impaired for Reasons Other than Shortness of Breath*	For example, musculoskeletal problem or chest pain.

ALPHA Trial

Participant ID :

Baseline Magnitude of Effort

____Grade 4	*Extraordinary*	Becomes short of breath only with the greatest imaginable effort. No shortness of breath with ordinary effort.
____Grade 3	*Major*	Becomes short of breath with effort distinctly submaximal, but of major proportion. Tasks performed without pause unless the task requires extraordinary effort that may be performed with pauses.
____Grade 2	*Moderate*	Becomes short of breath with moderate effort. Tasks performed with occasional pauses and requiring longer to complete than the average person.
____Grade 1	*Light*	Becomes short of breath with little effort. Tasks performed with little effort or more difficult tasks performed with frequent pauses and requiring 50-100% longer to complete than the average person might require.
____Grade 0	*No Effort*	Becomes short of breath at rest, while sitting, or lying down.
____W	*Amount Uncertain*	Subject's exertional ability is impaired due to shortness of breath, but amount cannot be specified. Details are not sufficient to allow impairment to be categorised.
____X	*Unknown*	Information unavailable regarding limitation of effort.
____Y	*Impaired for Reasons Other than Shortness of Breath.*	For example, musculoskeletal problems, or chest pain.

ALPHA Trial

Participant ID : | | | |

TRANSITION DYSPNEA INDEX

Change in Functional Impairment

Date Completed: D D M M Y Y Y Y

_____-3	*Major Deterioration*	Formerly working and has had to stop working and has completely abandoned some of usual activities due to shortness of breath.
_____-2	*Moderate Deterioration*	Formerly working and has had to stop working or has completely abandoned some of usual activities due to shortness of breath.
_____-1	*Minor Deterioration*	Has changed to a lighter job and/or has reduced activities in number or duration due to shortness of breath. Any deterioration less than preceding categories.
_____0	*No Change*	No change in functional status due to shortness of breath.
_____+1	*Minor Improvement*	Able to return to work at reduced pace or has resumed some customary activities with more vigour than previously due to improvement in shortness of breath.
_____+2	*Moderate Improvement*	Able to return to work at nearly usual pace and/or able to return to most activities with moderate restriction only.
_____+3	*Major Improvement*	Able to return to work at former pace and able to return to full activities with only mild restriction due to improvement of shortness of breath.
_____Z	*Further Impairment for Reasons Other than Shortness of Breath*	Subject has stopped working, reduced work, or has given up or reduced other activities for other reasons. For example, other medical problems, being "laid off" from work, etc.

ALPHA Trial

Participant ID :

Change in Magnitude of Task

____-3	*Major Deterioration*	Has deteriorated two grades or greater from baseline status.
____-2	*Moderate Deterioration*	Has deteriorated at least one grade but fewer than two grades from baseline status.
____-1	*Minor Deterioration*	Has deteriorated less than one grade from baseline. Subject with distinct deterioration within grade, but has not changed grades.
____0	*No Change*	No change from baseline.
____+1	*Minor Improvement*	Has improved less than one grade from baseline. Subject with distinct improvement within grade, but has not changed grades.
____+2	*Moderate Improvement*	Has improved at least one grade but fewer than two grades from baseline.
____+3	*Major Improvement*	Has improved two grades or greater from baseline.
____Z	*Further Impairment for Reasons Other than Shortness of Breath*	Subject has reduced exertion capacity, **but** not related to shortness of breath. For example, musculoskeletal problem or chest pain.

ALPHA Trial

Participant ID :

Change in Magnitude of Effort

____-3	*Major Deterioration*	Severe decrease in effort from baseline to avoid shortness of breath. Activities now take 50-100% longer to complete than required at baseline.
____-2	*Moderate Deterioration*	Some decrease in effort to avoid shortness of breath, although not as great as preceding category. There is greater pausing with some activities.
____-1	*Minor Deterioration*	Does not require more pauses to avoid shortness of breath, but does things with distinctly less effort than previously to avoid breathlessness.
____0	*No Change*	No change in effort to avoid shortness of breath.
____+1	*Minor Improvement*	Able to do things with distinctly greater effort without shortness of breath. For example, may he able to carry out tasks somewhat more rapidly than previously.
____+2	*Moderate Improvement*	Able to do things with fewer pauses and distinctly greater effort without shortness of breath. Improvement is greater than preceding category, but not of major proportion.
____+3	*Major Improvement*	Able to do things with much greater effort than previously with few, if any, pauses. For example, activities may be performed 50-100% more rapidly than at baseline.
____Z	*Further Impairment for Reasons Other than Shortness of Breath*	Subject has reduced exertional capacity, but not related to shortness of breath. For example, musculoskeletal problem or chest pain.

ALPHA Trial

Participant ID:

Dyspnea Recordings at Baseline (BDI)	Date:	Patient initials:	
	Functional Impairment (Types/kind of activities)	**Magnitude of Task** (Level, magnitude, extent)	**Magnitude of Effort** (Time needed, pauses, exertion)
Job			
House Work, Shopping			
Leisure Activities, Gardening			
Social Activities			
Washing/Dressing			
At Rest			
Any Other			
BDI Total Score:	Functional Impairment:	Magnitude of Task:	Magnitude of Effort:

Instruction: Describe activities still possible or given up and characterize under the three categories.

Appendix J - Case report form (CRF)

CASE REPORT FORM

Does allopurinol reduce right ventricular mass in lung disease associated pulmonary hypertension? The ALPHA Trial

Participant ID		Randomisation ID	

Visit		Date/ time	Taxi Required?	Taxi Booked?	Pre-visit reminder phone call	Comment
1 Screening	-1 to -4 weeks					
2 (a) Baseline/Randomisation	Day 0					
2 (b) MRI	+/- 2 weeks of baseline visit					
3 Progress	2 week (+/- 3 days)					
4 Progress	6 week (+/- 1 week)					
5 Tele Call	8 week (+/- 3 days)					
6 Progress	Month 3 (+/- 1 week)					
7 Tele call	Month 6 (+/- 2weeks)					
8 Progress	Month 9 (+/- 2 weeks)					
9 (a) Final visit	Month 12 (+/- 2 weeks)					
9 (b) MRI	Month 12 (+/- 2 weeks)					

ALPHA

Participant ID:

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ALPHA

Participant ID: | | | |

VISIT 1 CONSENT/ELIGIBILITY

INFORMED CONSENT

Has the subject given written informed consent? YES ☐ NO ☐

If YES, date of consent ☐☐-☐☐-☐☐☐☐

INCLUSION CRITERIA

	The following items MUST be answered YES for participant to be included in the trial	YES	NO
1	Participant is willing and able to give informed consent		
2	Aged over 18 years		
3	Previously diagnosed with COPD or ILD		
4	Screening echo based on diagnosis of PH as indicated by RVSP >25mmHg <u>and/or</u> PAT <110ms^{-1} <u>and/or</u> RV wall $\geq$5.5mm		
5	Stable lung disease medication for at least two weeks prior to consent		
6	Women of child bearing potential must agree to scheduled pregnancy testing prior to and during study treatment period and to use an appropriate method of contraception if sexually active.		

EXCLUSION CRITERIA

	The following criteria MUST be answered NO for participant to be included in the trial	YES	NO
1	Documented allergy or intolerance to allopurinol		
2	Objection to taking capsules made from animal sourced gelatine		
3	Left Ventricular Ejection Fraction <45% on echocardiography screening		
4	Severe aortic stenosis on echocardiography (screening)		

ALPHA Participant ID: | | | |

5	Active gout (i.e. flare up < 2 years) or currently taking allopurinol		
6	Severe hepatic disease		
7	Renal disease; CKD 3B or worse		
8	On azathioprine, 6-metacaptopurine or theophylline		
9	Malignancy (receiving active treatment) or other life threatening diseases		
10	Pregnant or lactating		
11	Contraindication to MRI (claustophobia, metal implants)		
12	Participated in any other clinical drug trial within the previous 30 days		
13	Any other considered by a study physician to be inappropriate for inclusion		

If any inclusion criteria is answered NO, or any exclusion criteria answered YES the participant is NOT eligible for the trial and must not be included in the study. Please list reason(s) for ineligibility for screen failure on Off Study Page.

Signed		Name		Date	

ALPHA

Participant ID: ☐☐☐

VISIT 1 SCREENING

PATIENT CHARACTERISTICS
Age ☐☐
Sex: Male ☐ Female ☐

PAST MEDICAL HISTORY	YES	NO
1. Chronic Obstructive Pulmonary Disease	☐	☐
2. Interstitial Lung Disease	☐	☐
3. Ischaemic Heart Disease (Angina / MI)	☐	☐
4. Hypertension	☐	☐
5. Hypercholesterolemia	☐	☐
6. Cerebrovascular disease (Stroke/TIA)	☐	☐
7. Diabetes mellitus	☐	☐
8. Others. If yes, please specify	☐	☐

CONCOMITANT MEDICATION (log updated)	YES ☐	NO ☐

AE/SAE (log updated)	YES ☐	NO ☐

SOCIAL HISTORY	
Smoking status: Current smoker ☐ Never ☐ Ex-smoker ☐ E-Cigarette ☐ Cigars ☐ Pipe ☐	
Pack years ☐☐☐	Cigarettes/day _____ Years smoked _____
Average weekly alcohol intake: ☐☐☐ Units	
Postcode: ☐☐☐☐	

ALPHA

Participant ID: ☐☐☐

HEIGHT
Height ☐ . ☐☐ *m*
WEIGHT
Weight ☐☐☐ . ☐ *kg*

VITAL SIGNS			
Blood Pressure (mmHg)	☐☐☐ / ☐☐☐	Pulse (BPM)	☐☐☐
O2 sats %	☐☐☐	Respiratory rate	☐☐

EXAMINATION		
NORMAL ☐	ABNORMAL (NOT SIGNIFICANT) ☐	ABNORMAL (SIGNIFICANT) ☐
Comments:		

Female of child bearing potential who is sexually active?	N/A ☐	YES ☐	NO ☐
URINE PREGNANCY TEST		YES ☐	NO ☐
RESULT		Positive ☐	Negative ☐

SCREENING ECHOCARDIOGRAPHY
LV Systolic function: 1. Normal/Mild (EF ≥ 45%) ☐ 2. Abnormal (EF ≤44%) ☐
Severe Aortic stenosis: Yes ☐ No ☐
RVSP > 25mmHg Yes ☐ No ☐ Not done ☐ ☐☐☐
PAT < $110ms^{-2}$ Yes ☐ No ☐ Not done ☐ ☐☐☐
RV wall ≥ 5.5mm Yes ☐ No ☐ Not done ☐ ☐☐ . ☐

SAFETY/BASELINE BLOODS	YES ☐	NO ☐

ALPHA Participant ID: | | | |

ADMINISTRATION	YES	NO
1. Patient given copy of consent	☐	☐
2. Screening log updated	☐	☐
3. Inform GP of screening failure if applicable N/A	☐	☐
4. CMRI safety checklist completed and sent to MRI (+copy of consent)	☐	☐

VISIT 2 BOOKED (record on front of CRF)	YES ☐	NO ☐

Signed		Name		Date	

DATA RECORDED IN OC	Signed ___________	Date___________	YES ☐	NO ☐

ALPHA

Participant ID: | | |

BLOOD RESULTS – VISIT 1

LAB TEST	Not done	RESULTS	UNITS	Action / Comments
Hb		☐☐☐	g/L	
HCT		☐.☐☐		

Bloods checked and signed YES ☐ NO ☐

Date of bloods ☐☐-☐☐-☐☐☐☐

Signed		Name		Date	

DATA RECORDED IN OC Signed __________ Date__________ YES ☐ NO ☐

ALPHA

Participant ID: | | | |

VISIT 2A / BASELINE - RANDOMISATION

PRE VISIT 2 PHONE CALL	
Confirm patient appointment time/date/transport	☐
Visit 1 baseline/safety bloods reviewed and recorded in bloods Log	☐

INCLUSION/EXCLUSION CRITERIA		
	YES	NO
Are all the **inclusion** criteria still met?		
Are all the **exclusion** criteria still met?		

AE/SAE (log updated) YES ☐ NO ☐

CONCOMITANT MEDICATION (log updated) YES ☐ NO ☐

OXYGEN USE
Is patient on long term oxygen? Yes ☐ No ☐

VITAL SIGNS			
Blood Pressure (mmHg)	☐☐☐ / ☐☐☐	Pulse (BPM)	☐☐☐
O2 sats %	☐☐☐	Respiratory rate	☐☐

Female of child bearing potential who is sexually active?	N/A ☐	YES ☐	NO ☐
URINE PREGNANCY TEST		YES ☐	NO ☐
RESULT	Positive ☐		Negative ☐

ALPHA

Participant ID: ☐☐☐

QUALITY OF LIFE QUESTIONNAIRES	YES	NO
1. SGRQ	☐	☐
2. SF-36	☐	☐
3. BDI-TDI	☐	☐
4. K-BILD (ILD only) N/A ☐	☐	☐

PULMONARY FUNCTION TEST	COMPLETED Yes ☐ No ☐
FEV_1 ☐ . ☐☐ L	Predicted ☐☐☐ %
FVC ☐ . ☐☐ L	Predicted ☐☐☐ %

RESEARCH BLOODS YES ☐ NO ☐

GENETIC BLOODS YES ☐ NO ☐

6 MINUTE WALK TEST	
Completed YES ☐ NO ☐	
Distance walked in 6 mins	☐☐☐ *m*
Pre- test O2 sats 6 min walk	☐☐☐ %
Post-test O2 sats 6 min walk	☐☐☐ %

RANDOMISATION	
Randomisation and log completed	YES ☐ NO ☐
Randomisation number	☐☐☐

ALPHA contact card to participant YES ☐ NO ☐

ALPHA

Participant ID: ☐☐☐

STUDY MEDICATION DISPENSED 100 mg/placebo	YES ☐	NO ☐

ADMINISTRATION	YES	NO
1. Letter to GP re enrolment	☐	☐
2. Consent form in notes	☐	☐
3. PIS/trial sticker/divider in notes	☐	☐
Any comments:		

VISIT 3 BOOKED (record on front of CRF)	YES ☐	NO ☐

Signed		Name		Date	

DATA RECORDED IN OC	Signed ___________	Date___________	YES ☐	NO ☐

ALPHA

Participant ID: ☐☐☐

VISIT 2B / MRI

MRI			
Has the subject had an MRI?	YES ☐	NO ☐	
Date of scan	☐☐ - ☐☐ - ☐☐☐☐		

Signed		Name		Date	

DATA RECORDED IN OC Signed ________________ Date__________ YES ☐ NO ☐

ALPHA

Participant ID: | | | |

VISIT 2C / Transfer Factor

Transfer Factor		
Has the subject had transfer factor measured?	YES ☐	NO ☐
Date of test	☐☐ - ☐☐ - ☐☐☐☐	
Result of DLCO	☐☐ . ☐	mmol/kPa.min
	☐☐☐	% predicted

Signed		Name		Date	

DATA RECORDED IN OC Signed _______________ Date__________ YES ☐ NO ☐

ALPHA

Participant ID: ☐☐☐

VISIT 3

PRE VISIT 3 PHONE CALL	
Confirm patient appointment time/date/transport	☐

AE/SAE (log updated)	YES ☐	NO ☐
CONCOMITANT MEDICATION (log updated)	YES ☐	NO ☐

ALLOPURINOL/PLACEBO COMPLIANCE

No. of tablets issued at last visit	☐☐☐	COMPLIANCE =
No. of tablets returned	☐☐☐	No. of drugs issued – No. of drugs returned
No. of drugs that should have been taken	☐☐☐	No. of drugs that should have been taken
% Compliance	☐☐☐	

VITAL SIGNS

Blood Pressure (mmHg)	☐☐☐ / ☐☐☐	Pulse (BPM)	☐☐☐
O2 sats %	☐☐☐	Respiratory rate	☐☐

URINE PREGNANCY TEST	N/A ☐	YES ☐	NO ☐
RESULT	Positive ☐		Negative ☐

SAFETY BLOODS AND URIC ACID	YES ☐	NO ☐
STUDY MEDICATION DISPENSED 300 mg/placebo If No, Down-titration/withdrawal form required	YES ☐	NO ☐
VISIT 4 BOOKED (record on front of CRF)	YES ☐	NO ☐

Signed		Name		Date	

DATA RECORDED IN OC Signed _______________ Date__________	YES ☐	NO ☐

ALPHA

Participant ID: ☐☐☐

BLOOD RESULTS – VISIT 3

LAB TEST	Not done	RESULTS	UNITS	Action / Comments
Hb		☐☐☐	g/L	
HCT		☐.☐☐		

Bloods checked and signed YES ☐ NO ☐

Date of bloods ☐☐-☐☐-☐☐☐☐

Signed		Name		Date	

DATA RECORDED IN OC Signed __________ Date__________ YES ☐ NO ☐

ALPHA

Participant ID:

VISIT 4

PRE VISIT 4 PHONE CALL	
Confirm patient appointment time/date/transport	☐
Visit 3 safety bloods reviewed and recorded in bloods Log	☐

AE/SAE (log updated)	YES ☐	NO ☐
CONCOMITANT MEDICATION (log updated)	YES ☐	NO ☐

ALLOPURINOL/PLACEBO COMPLIANCE

No. of tablets issued at last visit	☐☐☐
No. of tablets returned	☐☐☐
No. of drugs that should have been taken	☐☐☐
% Compliance	☐☐☐

COMPLIANCE =

$$\frac{\text{No. of drugs issued} - \text{No. of drugs returned}}{\text{No. of drugs that should have been taken}}$$

VITAL SIGNS

Blood Pressure (mmHg)	☐☐☐ / ☐☐☐	Pulse (BPM)	☐☐☐
O2 sats %	☐☐☐	Respiratory rate	☐☐

URINE PREGNANCY TEST	N/A ☐	YES ☐	NO ☐
RESULT	Positive ☐		Negative ☐

SAFETY BLOODS AND URIC ACID	YES ☐	NO ☐
STUDY MEDICATION DISPENSED 300 mg/placebo If NO, Down-titration/withdrawal form required:	YES ☐	NO ☐
VISIT 5 reminder (record on front of CRF)	YES ☐	NO ☐

Signed		Name		Date	

DATA RECORDED IN OC Signed ___________ Date___________ YES ☐ NO ☐

ALPHA

Participant ID: ☐☐☐

BLOOD RESULTS – VISIT 4

LAB TEST	Not done	RESULTS	UNITS	Action / Comments
Hb		☐☐☐	g/L	
HCT		☐.☐☐		

Bloods checked and signed YES ☐ NO ☐

Date of bloods ☐☐-☐☐-☐☐☐☐

Signed		Name		Date	

DATA RECORDED IN OC Signed __________ Date__________ YES ☐ NO ☐

ALPHA

Participant ID: ☐☐☐

VISIT 5 PHONE CALL

PRE VISIT 5 PHONE CALL	
Visit 4 safety bloods reviewed and recorded in bloods Log	☐

AE/SAE (log updated)	YES ☐	NO ☐
CONCOMITANT MEDICATION (log updated)	YES ☐	NO ☐
DRUG COMPLIANCE DISCUSSED	YES ☐	NO ☐
VISIT 6 BOOKED (record on front of CRF)	YES ☐	NO ☐
VISIT 5 COMPLETED	YES ☐	NO ☐

Signed		Name		Date	

DATA RECORDED IN OC Signed ___________ Date___________	YES ☐	NO ☐

ALPHA

Participant ID: | | |

VISIT 6

PRE VISIT 6 PHONE CALL	
Confirm patient appointment time/date/transport	☐

AE/SAE (log updated)	YES ☐	NO ☐
CONCOMITANT MEDICATION (log updated)	YES ☐	NO ☐

ALLOPURINOL/PLACEBO COMPLIANCE

No. of tablets issued at last visit	
No. of tablets returned	
No. of drugs that should have been taken	
% Compliance	

COMPLIANCE =

$$\frac{\text{No. of drugs issued} - \text{No. of drugs returned}}{\text{No. of drugs that should have been taken}}$$

VITAL SIGNS

Blood Pressure (mmHg)	/	Pulse (BPM)	
O2 sats %		Respiratory rate	

URINE PREGNANCY TEST	N/A ☐	YES ☐	NO ☐
RESULT	Positive ☐		Negative ☐

SAFETY BLOODS AND URIC ACID	YES ☐	NO ☐
STUDY MEDICATION DISPENSED 300 mg/placebo If No, Down-titration/withdrawal form required	YES ☐	NO ☐
VISIT 7 call reminder (record on front of CRF)	YES ☐	NO ☐

Signed		Name		Date	

DATA RECORDED IN OC	Signed ___________	Date___________	YES ☐	NO ☐

ALPHA

Participant ID:

BLOOD RESULTS – VISIT 6

LAB TEST	Not done	RESULTS	UNITS	Action / Comments
Hb			g/L	
HCT		.		

Bloods checked and signed	YES ☐ NO ☐
Date of bloods	- -

Signed		Name		Date	

DATA RECORDED IN OC Signed ___________ Date___________ YES ☐ NO ☐

ALPHA

Participant ID: | | |

VISIT 7 PHONE CALL

PRE VISIT 7	
Visit 6 safety bloods reviewed and recorded in bloods Log	☐

AE/SAE (log updated)	YES ☐	NO ☐
CONCOMITANT MEDICATION (log updated)	YES ☐	NO ☐
DRUG COMPLIANCE DISCUSSED	YES ☐	NO ☐
VISIT 8 BOOKED (record on front of CRF)	YES ☐	NO ☐
VISIT 7 COMPLETED	YES ☐	NO ☐

Signed		Name		Date	

DATA RECORDED IN OC Signed __________ Date__________	YES ☐	NO ☐

ALPHA

Participant ID: | | | |

VISIT 8

PRE VISIT 8 PHONE CALL	
Confirm patient appointment time/date/transport	☐

AE/SAE (log updated)	YES ☐	NO ☐

CONCOMITANT MEDICATION (log updated)	YES ☐	NO ☐

ALLOPURINOL/PLACEBO COMPLIANCE		
No. of tablets issued at last visit	☐☐☐	COMPLIANCE =
No. of tablets returned	☐☐☐	$\frac{\text{No. of drugs issued – No. of drugs returned}}{\text{No. of drugs that should have been taken}}$
No. of drugs that should have been taken	☐☐☐	
% Compliance	☐☐☐	

VITAL SIGNS			
Blood Pressure (mmHg)	☐☐☐ / ☐☐☐	Pulse (BPM)	☐☐☐
O2 sats %	☐☐☐	Respiratory rate	☐☐

URINE PREGNANCY TEST	N/A ☐	YES ☐	NO ☐
RESULT	Positive ☐		Negative ☐

SAFETY BLOODS AND URIC ACID	YES ☐	NO ☐

STUDY MEDICATION DISPENSED 300 mg/placebo If No, Down-titration/withdrawal form required	YES ☐	NO ☐

VISIT 9 BOOKED (record on front of CRF)	YES ☐	NO ☐

Signed		Name		Date	

DATA RECORDED IN OC Signed _______________ Date__________	YES ☐	NO ☐

ALPHA

Participant ID: ☐☐☐

BLOOD RESULTS – VISIT 8

LAB TEST	Not done	RESULTS	UNITS	Action / Comments
Hb		☐☐☐	g/L	
HCT		☐.☐☐		

Bloods checked and signed YES ☐ NO ☐

Date of bloods ☐☐-☐☐-☐☐☐☐

Signed		Name		Date	

DATA RECORDED IN OC Signed ___________ Date___________ YES ☐ NO ☐

ALPHA

Participant ID: ☐ ☐ ☐

VISIT 9A / FINAL VISIT

PRE VISIT 9 PHONE CALL	
Confirm patient appointment time/date/transport	☐
Visit 8 safety bloods reviewed and recorded in bloods Log	☐

AE/SAE (log updated)	YES ☐	NO ☐

CONCOMITANT MEDICATION (log updated)	YES ☐	NO ☐

OXYGEN USE		
Is patient is on long term oxygen?	Yes ☐	No ☐

ALLOPURINOL/PLACEBO COMPLIANCE		
No. of tablets issued at last visit	☐☐☐	COMPLIANCE =
No. of tablets returned	☐☐☐	
No. of drugs that should have been taken	☐☐☐	$\frac{\text{No. of drugs issued – No. of drugs returned}}{\text{No. of drugs that should have been taken}}$
% Compliance	☐☐☐	

VITAL SIGNS			
Blood Pressure (mmHg)	☐☐☐ / ☐☐☐	Pulse (BPM)	☐☐☐
O2 sats %	☐☐☐	Respiratory rate	☐☐

URINE PREGNANCY TEST	N/A ☐	YES ☐	NO ☐
RESULT	Positive ☐		Negative ☐

ALPHA

Participant ID:

QUALITY OF LIFE QUESTIONNAIRES	YES	NO
1. SGRQ	☐	☐
2. SF-36	☐	☐
3. BDI-TDI	☐	☐
4. K-BILD (ILD only) N/A ☐	☐	☐
If not done reason why:		

ECHOCARDIOGRAPHY				
RVSP > 25mmHg	Yes ☐	No ☐	Not done ☐	☐☐☐
PAT < 110ms^{-2}	Yes ☐	No ☐	Not done ☐	☐☐☐
RV wall ≥ 5.5mm	Yes ☐	No ☐	Not done ☐	☐☐.☐

PULMONARY FUNCTION TEST		COMPLETED Yes ☐ No ☐
FEV1 ☐ . ☐☐ %	Predicted ☐☐☐ %	
FVC ☐ . ☐☐ %	Predicted ☐☐☐ %	
Comment/reason if not done:		

SAFETY BLOODS AND URIC ACID	YES ☐	NO ☐

RESEARCH BLOODS	YES ☐	NO ☐

ALPHA

Participant ID: | | | |

6 MINUTE WALK TEST		
Completed	Yes ☐	No ☐
Distance walked in 6 mins		m
Pre- test O2 sats 6 min walk		%
Post-test O2 sats 6 min walk		%
Comments/ if not done reason why:		

END OF STUDY FORM COMPLETED YES ☐ NO ☐

Signed		Name		Date	

DATA RECORDED IN OC Signed _______________ Date__________ YES ☐ NO ☐

ALPHA

Participant ID:

BLOOD RESULTS – VISIT 9

LAB TEST	Not done	RESULTS	UNITS	Action / Comments
Hb			g/L	
HCT		.		

Bloods checked and signed YES ☐ NO ☐

Date of bloods __ __ - __ __ - __ __ __ __

Signed		Name		Date	

DATA RECORDED IN OC Signed ____________ Date____________ YES ☐ NO ☐

ALPHA

Participant ID: | | | |

VISIT 9B / MRI

MRI			
Has the subject had an MRI?	YES ☐	NO ☐	
Date of scan	☐☐ - ☐☐ - ☐☐☐☐		

Signed		Name		Date	

DATA RECORDED IN OC Signed ________________ Date___________ YES ☐ NO ☐

ALPHA

Participant ID: | | | |

6-MINUTE WALK TEST - BASELINE

6-Minute Walk Test - Baseline	
Completed	YES ☐ NO ☐
Date of test	☐☐ - ☐☐ - ☐☐☐☐

Pre- test HR	☐☐☐ beats/min
Post-test HR	☐☐☐ beats/min
Pre- test Dyspnoea Borg	☐☐☐
Post-test Dyspnoea Borg	☐☐☐
Pre- test Fatigue Borg	☐☐☐
Post-test Fatigue Borg	☐☐☐
Comments/ if not done reason why:	

Signed		Name		Date	

DATA RECORDED IN OC Signed __________ Date__________ YES ☐ NO ☐

ALPHA

Participant ID: | | |

6-MINUTE WALK TEST - FINAL

6-Minute Walk Test - Final		
Completed	YES ☐	NO ☐
Date of test	_ _ - _ _ - _ _ _ _	

Pre- test HR		beats/min
Post-test HR		beats/min
Pre- test Dyspnoea Borg		
Post-test Dyspnoea Borg		
Pre- test Fatigue Borg		
Post-test Fatigue Borg		

Comments/ if not done reason why:

Signed		Name		Date	

DATA RECORDED IN OC Signed __________ Date__________ YES ☐ NO ☐

ALPHA

Participant ID: | | | |

ALPHA BLOOD LOG

	Visit 1	Visit 3	Visit 4	Visit 6	Visit 8	Visit 9
Date						
Sodium (mmol/L)						
Potassium (mmol/L)						
Urea (mmol/L)						
Creatinine (mmol/L)						
eGFR (mL/min)						
Bilirubins (µmol/L)						
ALT (U/L)						
ALP (U/L)						
Albumin (g/L)						
Haemoglobin (g/L)						
Haematocrit						
WBC $x10^9$/L						
Platelets $x10^9$/L						
Blood glucose (mmol/L)						
Uric acid (mg/dL)						
HbA1C (mmol/mol)						
Cholesterol (mmol/L)						
HDL-C (mmol/L)						
Initial/Date						

ALPHA

Participant ID:

CONCOMITANT MEDICATIONS LOG

Name of Drug	* Drug Code See below for list	Dose	Units	Frequency	** Route of Admin, State if other	On-going at start of study (✓) or Start Date	On-going at end of study (✓) or Date Stopped or dose changed
						☐ __/__/____	☐ __/__/____
						☐ __/__/____	☐ __/__/____
						☐ __/__/____	☐ __/__/____
						☐ __/__/____	☐ __/__/____
						☐ __/__/____	☐ __/__/____
						☐ __/__/____	☐ __/__/____
						☐ __/__/____	☐ __/__/____
						☐ __/__/____	☐ __/__/____
						☐ __/__/____	☐ __/__/____

ALPHA

Participant ID: ☐☐☐

MEDICATION CODING			
1	Inhaled Corticosteroids (ICS)	14	α Blocker
2	Inhaled Long Acting Muscarinic Antagonist (LAMA)	15	Thiazide diuretic
3	Inhaled Long Acting Beta-Agonist (LABA)	16	Loop diuretic
4	ICS + LABA	17	Aldosterone antagonist
5	LAMA + LABA	18	ARB
6	Inhaled Short Acting Beta-Agonist (SABA)	19	Statin
7	Inhaled Short Acting Muscarinic Antagonist (SAMA)	20	Aspirin
8	Oral Steroid	21	Clopidogrel
9	Nebulisers	22	Warfarin
10	N-Acetylcysteine	23	Oral hypoglycaemic
11	ACE-I	24	Insulin
12	β Blocker	25	Other
13	Ca-channel blocker		

TASC ALPHA

Adverse Event Log		
Does Allopurinol reduce right ventricular mass in lung disease associated pulmonary hypertension?		
REC ID: 14/ES/1035	**Sponsor R&D Protocol ID:** 2013CV11	**EudraCT ID:** 2014-002302-38
Sponsor: University of Dundee – NHS Tayside	**Chief Investigator:** Prof A Struthers	**Principal Investigator:** Dr P Liu Shiu Cheong
Site: Tayside	**Participant ID:**	

Description of adverse event (provide additional information on notes pages if required)	**Date of onset**	**Date reported to Investigator /team**	**Severity** 1. Mild 2. Moderate 3. Severe	**Causality** 1. Unrelated 2. Possible 3. Probable 4. Definite	**Action taken – please list all that apply** 1. None 2. Hospitalisation 3. Intervention stopped 4.Con Meds commenced (record on Con Meds Log) 5. Other (specify)	**Outcome** 1. Recovered 2. Ongoing 3. Disability or incapacity 4. Death 5. Unknown	**Is this a Serious AE?** YES or NO	**If a SAE, is this reportable according to study protocol?** **YES/NO***	**Date resolved** (Enter date resolved/date of death or tick if ongoing at end of study)	**Signature and Date** PI or delegated doctor
								*complete an SAE form and email to the Sponsor pharmacovigilance.tayside@nhs.net		
	__/__/__	__/__/__							__/__/__ or ☐	
	__/__/__	__/__/__							__/__/__ or ☐	
	__/__/__	__/__/__							__/__/__ or ☐	
	__/__/__	__/__/__							__/__/__ or ☐	

ALPHA

Participant ID: [ ][ ][ ]

DOWN TITRATION/STOPPING MEDICATION

TO BE COMPLETED WHEN A PATIENT IS DOWN TITRATING OR MEDICATION STOPPED

What was the previous dose? 100mg ☐ 300mg ☐ 600mg ☐

Date of last dose [ ][ ]-[ ][ ]-[ ][ ][ ][ ]

Has the subject returned any tablets YES* ☐ NO ☐

*If YES, how many [ ][ ]

DOWN TITRATING

Reason for Down-Titrating *(tick at least one):*

Rash / Adverse Event ☐

Abnormal blood result ☐

What is the new dose? 100mg ☐ 300mg ☐ None/medication stopped ☐

Number of tablets given

STOPPING MEDICATIONS

Reason for stopping *(tick at least one):*

Rash ☐

Other adverse event ☐

Participant choice ☐

Other (please specify) ☐

Signed		Name		Date	

ALPHA

Participant ID: ☐☐☐

COMPLETION OF STUDY/ WITHDRAWAL

COMPLETION

Did the participant complete the study? YES ☐ NO ☐

Date of completion/withdrawal ☐☐-☐☐-☐☐☐☐

If subject did not complete study give reason:

Adverse event ☐

Contraindication to MRI ☐

Lack of compliance to medication ☐

Participant withdrawal ☐

Death ☐

Other, please specify ☐

FOLLOW UP

Is any follow-up required? YES ☐ NO ☐

IF YES, please provide details:

PROTOCOL

Were there any deviations from protocol? YES ☐ NO ☐

If YES, provide details and note in Trial Master File and Protocol Deviation Log:

Signed		Name		Date	